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# THE INFLUENCE OF DEXTROSE INGESTION ON AMINO ACID NITROGEN, UREA NITROGEN AND HEMOGLOBIN CONCENTRATION OF THE BLOOD*

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DURING the last few years we have had occasion to study the influence of infection upon the tolerance for dextrose.[1] In addition a comparative study of dextrose and sucrose tolerance tests in normal and hospitalized subjects has been made.[2] Since little information is available as to the effect of ingested dextrose upon the amino acid and urea content of the blood in health and in disease, the sugar tolerance studies were expanded in order to secure data on these points also. In addition we found it both interesting and necessary to study the changes in blood volume, as measured by hemoglobin determinations, during the course of the tolerance tests.

In an experiment upon a normal human subject Folin and Berglund[3] found that the ingestion of glucose depresses the postabsorptive level of amino acids, urea, and nonprotein nitrogen in the blood. Katayama[4] reported that the ingestion of glucose by 5 subjects resulted in a fall in blood nonprotein nitrogen in two cases and an increased output of urine nitrogen whereas in two other cases the blood nonprotein nitrogen rose with a fall in the urinary nitrogen. According to Milheiro[5] the amino acid nitrogen of the blood decreases after eating. Bruger and Mirsky[6] performed 60 dextrose tolerance tests on 54 unselected subjects. They state: "(a) The urea nitrogen content of the blood may rise or (and) fall or remain unchanged. A gradual fall in

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the urea nitrogen (1 to 7 mg. per cent) was observed in 22 out of 45 cases. A definite decrease (3 mg. per cent or more) occurred more often in subjects with diminished carbohydrate tolerance than in those with normal tolerance. (b) The total nonprotein nitrogen of the blood may rise or (and) fall or show no marked change. . . ." Eveleth[7] found that the amino acid nitrogen content of the blood of pigs rose following glucose ingestion. In addition the urea usually increased although the average values showed practically no change.

The amino acids of the blood have been shown to be sensitive, also, to the injection of insulin. Thus Luck, Morrison, and Wilbur[8] proved that the injection of insulin lowers the amino acid nitrogen content of the blood of rabbits, rats, and human beings. Similar observations are reported by other workers.[9-31]

## THE INFLUENCE OF INGESTED DEXTROSE ON THE AMINO ACID NITROGEN AND UREA NITROGEN CONTENT OF THE BLOOD

*Methods.*—About 6 or 7 c.c. of blood was secured by venipuncture and transferred to a small glass jar containing 10 mg. of dry, finely divided potassium oxalate. The protein-free filtrate was immediately prepared according to Haden.[32] The blood sugar was then determined according to Benedict's method,[33] the urea nitrogen, with some modifications,[34] by direct nesslerization.[35, 36] The amino acid nitrogen content of the blood filtrates was determined by the colorimetric method of Folin[37] using sodium beta-naphthoquinone 4-sulphonate.[38] During the time that the present work was in progress Folin's method has been subjected to considerable criticism both favorable and unfavorable[39-47] and has undergone important modifications.[48-50] Hence considerable uncertainty exists at present regarding the actual amount of amino acid nitrogen in the blood under normal, experimental and pathologic conditions. We were not interested primarily in the absolute amount of amino acid nitrogen in the blood but in the relative quantities which were present during the course of the dextrose tolerance tests. For this purpose Folin's original method proved very satisfactory.

The question whether fasting per se or frequent removal of blood influences the amino acid nitrogen level seems to be somewhat in dispute.[10, 15, 18, 19, 51-55] Therefore we decided to run some control experiments on fasting patients. After the customary overnight fast 6 to 8 c.c. of blood was collected in the usual manner at hourly intervals during the morning. The patient ate nothing during this period although in some cases 300 c.c. of water was given to drink at 8 A.M. Blood sugar, urea nitrogen, amino acid nitrogen, uric acid, and hemoglobin were determined on each sample. Thus these control determinations were made at the same time intervals as when the various blood constituents were determined during the tolerance tests. The average values which are given at the foot of Table I prove decisively that no appreciable variations in these constituents occur during the course of the morning following a twelve-hour fast, nor are they in-

fluenced by frequent blood withdrawals. Our findings do not substantiate the conclusions recently drawn by Paschkis.[51]

## COMMENT

The general procedure followed in the administration of the dextrose has been described elsewhere.[1, 2] Eighty-seven individuals in all have been subjected to dextrose tolerance tests. In order to conserve space the data on each individual case have been omitted. The cases have been separated into 5 groups or classifications: 16 essentially normal individuals; miscellaneous diseases, 21 cases; arthritis and rheumatoid conditions, 12 cases; infection, 19 cases; diabetes mellitus, 19 cases. In this manner, beginning with the normal curves in the first group, the blood sugar values become more pathoglycemic until the hyperglycemia is also accompanied by the glycosuria characteristic of diabetes mellitus in the last group. The average value for the blood sugar, amino acid nitrogen and urea nitrogen determinations at each period of the test have been summarized (Table II).

An examination of the above summarized data plainly indicates that an appreciable consistent decrease in amino acid nitrogen and urea nitrogen occurs during the course of a glucose tolerance test. The decrease in these nitrogenous blood constituents seems to be independent of the nature of the disease or of the degree of hyperglycemia. Thus the average maximum decrease in amino acid nitrogen for each group of diseases which occurred during the course of the tests is as follows: normal cases 12.91 per cent, miscellaneous diseases 9.55 per cent, arthritis and rheumatoid conditions 10.22 per cent, infection 14.68 per cent, and diabetes mellitus 11.51 per cent. These values check fairly well with the average maximum decrease in amino acid nitrogen (12.91 per cent) which occurred in the blood of normal individuals following the ingestion of glucose. The average maximum decrease in this constituent for all 87 cases was 11.78 per cent. In addition it can readily be seen that the greater decrease in amino acid nitrogen occurred during the first hour of the test period, 53.0 per cent; 32.6 per cent of the decrease took place during the second hour, and 14.4 per cent during the third hour of the tolerance test. There seems to be no correlation between the nature of the disease or the degree of hyperglycemia and the decrease in the amino acid nitrogen produced by the ingestion of dextrose.

As regards the urea nitrogen changes following dextrose ingestion quite similar results were secured. Thus the average maximum decrease in blood urea nitrogen which occurred in each group of cases following the administration of dextrose is as follows: normal cases 13.57 per cent, miscellaneous diseases 13.70 per cent, arthritis and rheumatoid conditions 14.78 per cent, infection 15.02 per cent, and diabetes mellitus 11.00 per cent. Again there seems to be no significantly distinguishable difference between the percentage decrease in urea nitrogen for the group of normals and for the various groups of pathologic cases. The average maximum decrease in urea nitrogen for the 87 cases was 13.04 per cent, practically the same as that noted for the amino acid nitrogen (11.78 per cent). The greater decrease in the urea

TABLE I

VARIATIONS IN THE BLOOD CONSTITUENTS DURING THE FASTING CONTROL PERIOD

| WARD NUMBER | TIME A.M. | BLOOD SUGAR MG./100 C.C. | UREA NITROGEN MG. PER 100 C.C. | | | AMINO ACID NITROGEN MG./100 C.C. | URIC ACID MG./100 C.C. | HEMOGLOBIN PER CENT | DIAGNOSIS |
|---|---|---|---|---|---|---|---|---|---|
| | | | A | B | AVERAGE | | | | |
| K 8 | 8 | 91 | 7.9 | 7.9 | 7.9 | 5.9 | 3.33 | 100.0 | Streptococcal bronchitis |
| | 9 | 94 | 8.0 | 7.7 | 7.9 | 5.8 | 3.33 | 93.0 | |
| | 10 | 90 | 7.6 | 7.0 | 7.3 | 5.9 | 3.44 | 96.6 | |
| | 11 | 94 | 7.7 | 7.8 | 7.8 | 5.8 | 3.48 | 94.4 | |
| K 6 | 8 | 93 | 16.6 | 16.2 | 16.4 | 6.3 | 3.64 | 100.0 | Chronic nephritis with hypertension |
| | 9 | 92 | 17.2 | 15.8 | 16.5 | 6.4 | 3.56 | 102.0 | |
| | 10 | 90 | 16.8 | 16.1 | 16.5 | 6.4 | 3.51 | 100.0 | |
| | 11 | 94 | 16.0 | 15.3 | 15.7 | 6.1 | 3.69 | 98.5 | |
| A 21 | 8 | 95 | 8.6 | 8.0 | 8.3 | 5.8 | 3.81 | 100.0 | Acute bursitis |
| | 9 | 96 | 8.3 | 8.0 | 8.2 | 5.9 | 3.64 | 100.0 | |
| | 10 | 98 | 8.2 | 7.6 | 7.9 | 5.9 | 3.64 | 101.5 | |
| | 11 | 99 | 8.3 | 8.3 | 8.3 | 6.0 | 3.86 | 98.0 | |
| A 5 | 8 | 95 | 12.3 | 12.1 | 12.2 | 6.4 | | 100.0 | Gonococcal arthritis |
| | 9 | | | | | | | | |
| | 10 | 91 | 12.8 | 12.4 | 12.6 | 6.4 | | 98.5 | |
| | 11 | | 12.6 | | 12.6 | 6.5 | | 100.0 | |
| A 10 | 8 | 102 | 8.6 | 8.4 | 8.5 | 6.5 | 4.70 | 100.0 | Tinea saginita |
| | 9 | 104 | 8.5 | 8.1 | 8.3 | 6.3 | 4.85 | | |
| | 10 | 104 | 8.3 | 8.3 | 8.3 | 6.5 | 4.85 | 101.5 | |
| | 11 | 95 | 8.1 | 8.3 | 8.2 | 6.4 | 4.70 | 102.0 | |

TABLE I—CONT'D

| WARD NUMBER | TIME A.M. | BLOOD SUGAR MG./100 C.C. | UREA NITROGEN MG. PER 100 C.C. | | | AMINO ACID NITROGEN MG./100 C.C. | URIC ACID MG./100 C.C. | HEMOGLOBIN PER CENT | DIAGNOSIS |
|---|---|---|---|---|---|---|---|---|---|
| | | | A | B | AVERAGE | | | | |
| O 10 | 8 | 99 | 10.2 | 10.6 | 10.4 | 6.4 | 3.46 | | Prostatitis |
| | 9 | 99 | 9.9 | 10.4 | 10.2 | 6.2 | 3.70 | | |
| | 10 | 102 | 9.9 | 10.3 | 10.1 | 6.3 | 3.65 | | |
| | 11 | 105 | 10.2 | 10.2 | 10.2 | 6.2 | 3.52 | | |
| A 9 | 8 | 91 | 14.2 | 13.6 | 13.9 | 6.8 | 4.00 | 100.0 | Cerebral hemorrhage (apoplexy) |
| | 9 | 89 | 13.8 | 14.0 | 13.9 | 6.5 | 4.20 | 99.4 | |
| | 10 | 91 | 14.3 | 13.5 | 13.9 | 6.8 | 4.20 | 96.7 | |
| | 11 | 90 | 14.0 | 13.8 | 13.9 | 6.8 | 4.10 | 98.1 | |
| A 1 | 8 | 91 | 23.1 | 24.2 | 23.7 | 5.4 | | 100.0 | Chronic arthritis |
| | 9 | 87 | 23.4 | 23.4 | 23.4 | 5.6 | | 104.0 | |
| | 10 | 90 | 23.1 | 23.1 | 23.1 | 5.4 | | 102.0 | |
| | 11 | 89 | 23.1 | 23.9 | 23.5 | 5.5 | | 99.6 | |
| Average of above | | | | | | | | | |
| | 8 | 94.6 | | | 12.66 | 6.19 | 3.83 | 100.0 | |
| | 9 | 93.6 | | | 12.63 | 6.10 | 3.88 | 99.7 | |
| | 10 | 94.5 | | | 12.46 | 6.20 | 3.88 | 99.6 | |
| | 11 | 94.9 | | | 12.53 | 6.17 | 3.89 | 98.7 | |

TABLE II

| NUMBER OF CASES | CONDITION OF PATIENTS | BLOOD SUGAR MG./100 C.C. | | | | AMINO ACID N. MG./100 C.C. | | | | MAXIMUM DIFFERENCE PER CENT | UREA N. MG./100 C.C. | | | | MAXIMUM DIFFERENCE PER CENT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F. | 1 HR. | 2 HR. | 3 HR. | F. | 1 HR. | 2 HR. | 3 HR. | | F. | 1 HR. | 2 HR. | 3 HR. | |
| 16 | Normal cases | 99.4 | 140.9 | 113.7 | 95.4 | 6.35 | 6.00 | 5.69 | 5.83 | 12.91 | 11.57 | 10.74 | 10.40 | 10.00 | 13.57 |
| 21 | Misc. diseases | 100.1 | 163.8 | 137.9 | 113.8 | 6.28 | 5.93 | 5.71 | 5.68 | 9.55 | 13.50 | 12.55 | 11.65 | 12.06 | 13.70 |
| 12 | Arthritis, etc. | 102.1 | 197.1 | 177.2 | 136.8 | 6.26 | 5.79 | 5.62 | 5.67 | 10.22 | 10.42 | 10.19 | 9.13 | 8.88 | 14.78 |
| 19 | Infection | 107.6 | 205.2 | 191.6 | 137.7 | 6.20 | 5.73 | 5.49 | 5.29 | 14.68 | 11.05 | 10.20 | 9.67 | 9.39 | 15.02 |
| 19 | Diabetes mellitus | 117.5 | 232.3 | 225.8 | 174.8 | 6.34 | 6.04 | 5.80 | 5.61 | 11.51 | 10.59 | 10.18 | 9.53 | 9.35 | 11.00 |
| 87 | Average of above | | | | | 6.29 | 5.90 | 5.66 | 5.55 | 11.78 | 11.43 | 10.77 | 10.08 | 9.94 | 13.04 |
| | Average hourly decrease (milligrams) | | | | | | 0.39 | 0.24 | 0.106 | | | 0.66 | 0.69 | 0.14 | |
| | Average rate of decrease (per cent) | | | | | | 53.0 | 32.6 | 14.4 | | | 44.3 | 46.3 | 9.4 | |

nitrogen for all the cases occurred during the first and second hour of the test, namely 44.3 per cent and 46.3 per cent, respectively, and 9.4 per cent for the third hour period, whereas the greater decrease in amino acid nitrogen occurred during the first hour period. There seems to be no correlation between the disease or condition of the patient, the degree of hyperglycemia attained during the course of the test, and concomitant glycosuria if any, and the decrease in urea nitrogen.

In the later part of the present work data are given on the blood volume changes, as measured by relative hemoglobin determinations, which occurred during the course of these tests. Disregarding individual variations no consistent change in blood volume was noted despite the degree of hyperglycemia. Apparently the consistent decrease in amino acid nitrogen and urea nitrogen which we found to follow the alimentary ingestion of dextrose cannot be attributed to mere blood dilution.[56] However, it seems reasonable to believe that the decrease in these nitrogenous constituents of the blood observed during the course of the tolerance tests is due to the protein-sparing action[57] of the ingested dextrose. While the injection of insulin also results in a decrease in the amino acid nitrogen content of the blood, it is accompanied by an increase in blood urea. Since alimentary dextrose depresses both the amino acid and the urea content of the blood, it seems apparent that different metabolic factors are involved in each case. This is probably the explanation for the fact that simultaneous administration of glucose with insulin does not prevent the fall in blood and tissue amino acid nitrogen noted by Luck and Spaulding[24] and Ralli and Tiber.[25]

## ALIMENTARY HYPERGLYCEMIA, HEMOGLOBIN CONCENTRATION AND THE BLOOD VOLUME

The ability of the blood to maintain its constant internal environment has long been recognized. Changes in the water content of the blood under various experimental conditions have been frequently studied.[58-80] In order to eliminate the possibility that the definite decrease in amino acid nitrogen and urea nitrogen, which we found to take place during the course of a sugar tolerance test, might be due to dilution of the blood, the accompanying study of relative blood volume changes was made.

*Hemoglobin Determinations—Accuracy of the Method.*—A mixed, pooled sample of blood containing 10 mg. of potassium oxalate per 6 c.c. of blood was shaken with a rotary motion and the well-mixed blood drawn into a 0.5 c.c. Ostwald pipette. The blood was then discharged into a 500 c.c. Erlenmeyer flask containing exactly 250 c.c. of 0.1 N HCl. In this manner 10 samples of the same blood were set up for hemoglobin determinations. The flasks were shaken several times during the morning and then allowed to stand at room temperature for about twenty-four hours when colorimetric comparisons were made. The preparations were allowed to stand for this length of time in order to permit the development of a constant amount of color. The colorimeter was first carefully adjusted with the contents of flask No. 1 which was used as a standard. Each of the nine remaining flasks was

matched against this sample. Five readings were taken for each determination, none differing by more than 0.5 mm. from each other. The greatest variation from the specimen which was used as a standard and set at 20 mm. was 0.3 mm. and the maximum variation between the ten determinations was 0.5 mm. By this method, of course, only the relative concentration of hemoglobin can be estimated.

*Hemoglobin Concentration—Diurnal Variations.*—A number of experiments were also performed to see whether appreciable variations in hemoglobin concentration occur in subjects during the course of the day. About 3 or 5 c.c. of the blood was taken from the patient by venepuncture at 9 A.M., 12 Noon, and 4 P.M. Hemoglobin determinations were made in triplicate as previously outlined. After twenty-four hours the 9 A.M. preparation was used as a standard and the specimens collected at 12 Noon and 4 P.M., were matched against it. Thirty-four individual patients were studied in this manner.

The detailed findings have been omitted. However, the data indicate that appreciable variations in the concentration of hemoglobin occur in individual subjects during the course of the day. On the other hand, the relative average hemoglobin concentration of all the patients showed a slight decrease of doubtful significance as the day advanced. Assuming the relative average hemoglobin concentration to be 100 per cent at 9 A.M., it was 99.9 per cent at 12 noon and 98.57 per cent at 4 P.M. The average decrease, therefore, amounts to 0.1 per cent and 1.43 per cent for the noon and evening periods, respectively.

Six cases out of thirty-four 12 noon specimens differed by more than 0.5 mm., or 2.5 per cent in hemoglobin, from their corresponding morning specimens. The maximum decrease in hemoglobin for any one subject was 8.76 per cent and the maximum increase noted in any case was 5.3 per cent during this period. Among the 4 P.M. samples, ten of the thirty-four determinations differed by more than 0.5 mm. or 2.5 per cent in hemoglobin, from their corresponding morning specimens. The greatest relative decrease in hemoglobin concentration during this period was 10.07 per cent and the maximum increase in any one case was 5.8 per cent. The greatest variation which occurred in any one patient during the day was 10.07 per cent. These results are essentially in agreement with those of Dreyer, Bazette and Pierce,[81] Rabinowitch,[82] and Chanutin, Smith, and Mendel.[83] From these observations we can conclude that, although variations as high as 10.07 per cent in hemoglobin concentration occur during the course of the day, the average diurnal variation in hemoglobin concentration, or blood volume, was insufficient to account for the consistent decrease in amino acid nitrogen and urea nitrogen observed during the course of the sugar tolerance tests.

*Hemoglobin Concentration—Variations During the Course of Dextrose Tolerance Tests.*—In a similar manner hemoglobin determinations were made on the blood specimens used for the preparation of the protein-free filtrates. These experiments were performed in order to determine whether the osmotic

effects of the glucose in the stomach and later in the blood would exert an appreciable influence upon the blood volume as measured by variations in hemoglobin concentration.[56, 58, 84, 90]

Our experimental data were divided into two groups, one containing the findings on nineteen dextrose tolerance tests which yielded normal blood sugar curves and the other the findings on thirty-five tests which yielded high, diabetic, or diabetic-like blood sugar curves due to infection, diabetes mellitus or arthritis or allied rheumatoid conditions. The conclusions only will be reported. No consistent variation in the blood volume, as measured by relative hemoglobin percentages, occurred regardless of the degree of glycemia developing during the course of the tolerance tests. Thus the average blood sugar values for the normal curves at the fasting, one, two, and three hour periods of the tests were 98.0, 141.4, 109.2, and 98.8 mg. per 100 c.c. of blood, respectively. The corresponding average hemoglobin readings were 20.0 (set as standard), 19.97, 20.21, and 20.15 mm. or 100.0, 100.15, 98.96, and 99.26 per cent hemoglobin. The average blood sugar values for the pathoglycemic curves at each period of the test were 105.8, 206.5, 192.2, and 146.7 mg. per 100 c.c. of blood. The corresponding average hemoglobin readings were 20.0 (set as standard), 19.95, 20.11, and 20.20 mm. or 100.0, 100.26, 99.45, and 99.01 per cent hemoglobin. Obviously, therefore, no regular significant variation in blood volume occurred during the course of the dextrose tolerance tests regardless of the degree of hyperglycemia. Hence the decrease in nitrogenous constituents of the blood (amino acids and urea) following the ingestion of dextrose is not due to blood volume changes.

## SUMMARY

1. Eighty-seven individual cases have been subjected to dextrose tolerance tests during which the concentration of dextrose, amino acid nitrogen, urea nitrogen and hemoglobin of the blood have been determined. The data indicate that an appreciable decrease in the amino acid nitrogen and urea nitrogen content of the blood occurs during the course of dextrose tolerance tests. The cases have been separated into five groups. The average maximum decrease in amino acid nitrogen for each group was: normal cases 12.91 per cent, miscellaneous diseases 9.55 per cent, arthritis and rheumatoid conditions 10.22 per cent, infection 14.68 per cent, and diabetes mellitus 11.51 per cent. The average maximum decrease in urea nitrogen for these same groups was: normal cases 13.57 per cent, miscellaneous diseases 13.70 per cent, arthritis and rheumatoid conditions 14.78 per cent, infection 15.02 per cent, and diabetes mellitus 11.00 per cent. The average maximum decrease in urea nitrogen for the 87 cases was 13.04 per cent, practically the same as the average maximum decrease in amino acid nitrogen (11.78 per cent). No significant variations were noted between the results for the normal group and the pathologic groups. No correlation was observed between the various diseases or the degree of hyperglycemia attained during the course of the tests and the decrease in amino acid nitrogen and urea nitrogen content of the blood.

2. The concentration of blood dextrose, amino acid nitrogen, urea nitrogen, uric acid, and hemoglobin of control patients remained unchanged during the morning following a twelve-hour fast, despite hourly withdrawals of blood.

3. Diurnal variations in hemoglobin concentration up to 10 or 11 per cent were noted. The average values remained constant however. No relation between the degree of hyperglycemia and blood volume changes as determined by the relative hemoglobin concentration was noted during course of the glucose tolerance tests. Except for individual variations, the blood volume or hemoglobin concentration remained unchanged during the tests.

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# A COMPARATIVE STUDY OF GLUCOSE AND SUCROSE TOLERANCE TESTS*

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THE glucose tolerance test, or perhaps more correctly the blood sugar time curve[1] (1933), has acquired justified popularity, since it is the most sensitive test available for the detection and evaluation of disorders of carbohydrate metabolism. While the metabolism of glucose both under normal and pathologic conditions has been studied extensively, little attention has been given to sucrose metabolism, especially of the hospitalized patient.

Although glucose is utilized in every modern hospital, particularly for patients requiring intravenous therapy, it is not so readily available to the practicing physician, particularly those in the smaller communities. Cane sugar, sucrose, however, may be readily obtained pure and is much less expensive. In view of these facts a comparative study of the blood sugar curves, and sugar excretion if any, was made on a series of 57 hospital patients after the ingestion of glucose, followed a few days later by an equal quantity of sucrose, or vice versa.

## HISTORICAL RÉSUMÉ

The literature on sucrose metabolism is not voluminous. According to Röhman and Nagano[2] (1907) sucrose is rapidly hydrolyzed in the alimentary canal into its two component constituents glucose and fructose which upon absorption, produce a rise in the blood sugar. Similar observations have been made by von Noorden[3] (1917), Jacobsen[4] (1913), Eisner and Forster[5] (1921) and Frank and Mehlhorn[6] (1920). Whereas Worm-Muller[7] (1884) noted sucrose in the urine after the administration of 50 to 250 gm. of this sugar, other workers[6] usually report glucose as the sugar being present. Schatti[8] (1923) found that the blood sugar of normal individuals, who had ingested 20 gm. of cane sugar, rose as rapidly as with glucose or levulose although to a lesser height. Field[9] (1919) fed 100 gm. of sucrose to normal colored males. The average rise in blood sugar was 20 mg. per 100 c.c. Moultier and Camus[10] (1929) have compared glucose and sucrose tolerance tests in one diabetic and in six nondiabetic individuals. Puscko[11] (1931) has studied the influence on diabetic patients of injections of sucrose and invert sugar upon ketonuria, ketonemia, and intravenously injected insulin. Greenwald and Pennell[12] (1930) noted that in a series of 15 infants (two to ten days old) when fed 2 gm. of dextrose per kilo body weight all but one gave a prompt

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and definite rise in blood sugar. In only two infants, however, were maximum values of 140 mg. or above obtained by the ingestion of an equal amount of sucrose.[13] Utter[14] (1928) has studied the sugar excretion by children upon sucrose ingestion. He found that concentrated sucrose solutions produced a marked excretion of this sugar but that the same amount of sucrose in a more dilute solution was accompanied by less sucrosuria. Oppel and Fedorow[15] (1928) have studied the effects of sucrose ingestion in a series of 58 children. They found that the beginning period of the rise in the blood sugar coincided, in general, with the rise after glucose. The peak of the curve usually occurred within thirty minutes after the feeding. In a considerable number of experiments but a slight rise in blood sugar was found and, as a rule, the peak rarely exceeded 125 mg. per 100 c.c. In addition they noted the presence of a secondary and even an occasional tertiary rise in the blood sugar with a prolonged period of hyperglycemia. Gilbert, Schneider and Bock[17] (1926) report a secondary rise in blood sugar after glucose ingestion. In a further study with rabbits Oppel[18] (1929) introduced 2 to 4 gm. of sucrose per kilo of body weight by means of a stomach tube. Glycemia with a slight fructosemia (not exceeding 12 mg. per 100 c.c. of blood) was noted after the blood filtrates had been hydrolyzed with N/2 sulphuric acid. Sucrosemia, however, was not found. Reinhold and Karr[19] (1927) found that rabbits showed a moderate hyperglycemia from sucrose which had been administered by stomach tube. Ciaccio[20] (1927) found that depancreatized dogs yielded hyperglycemic blood sugar curves after the administration of cane sugar. The effect of sucrose upon the metabolism of dextrose and other sugars has been studied by Solarino[21] (1927), Ciaccio and Racchiusa[22] (1926-27), Merlino[23] (1930) and Lewis and Bond[24] (1930). Abderhalden and Buadze[25] (1932) state that dogs excrete the enzyme sucrase upon parenteral administration of sucrose. During recent years the laboratories of some of the various insurance companies have begun the study of sucrose metabolism with a view toward its substitution for glucose in tolerance tests.[26-30]

From this brief résumé of the prevailing literature it is evident that little information is available at present upon the metabolism of sucrose in health and in disease. This is particularly true in regard to hospital patients or individuals in whom a disturbance in carbohydrate metabolism has been produced by factors such as diabetes mellitus, renal glycosuria, arthritis, infections, particularly those of a pyogenic nature, hepatic disorders, endocrine diseases, etc.

It seems clear, from a review of the available work on blood sugar curves following sucrose ingestion by normal healthy individuals, that these curves are well within the normal limits as established by glucose tolerance tests. In fact, in most of the experimental studies the maximum blood sugar peak appears to be lower for sucrose than it is for glucose, and usually returns more rapidly to the normal fasting level.[31, 32]

The present work was undertaken in view of the fact that little information is available as to the type of blood sugar curve which may be given by

the general hospital patient upon the ingestion of sucrose. Furthermore few records are available where both sucrose and glucose tolerance tests were performed upon the same patient.

## PROCEDURE AND METHODS

The experimental procedure and methods are essentially the same as those which were used by the authors in the study of infection and the glucose tolerance[33] (1934). One and five-tenths grams of glucose (Merck or Pfanstiehl), or sucrose (Pfanstiehl or Crystal Domino) per kilogram of body weight, generally about 100 gm., was used for each test. Nausea was not observed. Since smoking per se produces a rise in blood sugar[34] (1931), the patients were instructed to refrain from the consumption of tobacco in any form during the course of the test. In general the patients were on the usual hospital diet except those patients who required special dietary treatments. The works of Hamman and Hirshman[35] (1919), McLean and de Wesselow[36] (1920-21), Foster[37] (1923), and du Vigneaud and Karr[38] (1925), etc., indicate the fact that a second dose of sugar, given after the hyperglycemia due to the first dose has disappeared, gives little if any rise in the blood sugar. Consequently a period of at least two days was allowed to elapse between the time of the first and the second tolerance tests. During this interval the patients returned to their usual diet. In some cases the glucose was given first, whereas in other cases the sucrose was given precedence. On the other hand, the time interval between the tests had to be sufficiently short so that no appreciable change could occur in the clinical condition of the patient.

The oxalated blood specimens were deproteinized according to the method of Folin-Wu[39] (1919), as modified by Haden[40] (1923), and the blood sugar determined by the Benedict[41] (1926) method.

*Analysis of Sucrose in the Blood.*—In a certain number of cases a test was made for unhydrolyzed sucrose in addition to the total reducing substances in the blood. Since sucrose per se does not reduce alkaline copper reagents, a preliminary hydrolysis is required. Although the experimental data has been omitted from this report, neither sucrose in the blood, sucrosemia, nor sucrose in the urine, sucrosuria, could be demonstrated regardless of the condition of the patient or the degree of hyperglycemia or glycosuria. The question whether sucrose may actually have been present in the blood stream during the course of the test, but had undergone inversion while the blood was being transported to the laboratory and deproteinized could possibly arise. This interval rarely exceeded thirty minutes, however. Although Berg[42] (1931) reports negative results, Abderhalden and Buadze[25] (1932) seem to have demonstrated decisively that invertase (sucrase) slowly appears in the blood and urine upon parenteral administration of cane sugar.

Under our experimental conditions, however, we have been unable to detect sucrase activity in ordinary fasting blood. Incubation of blood with sucrose did not result in an increase in reducing blood sugar. Hence inversion of the disaccharide had not taken place. The results are not surprising in view of the fact that intravenously injected sucrose is excreted as a for-

TABLE I

COMPARISON OF GLUCOSE AND SUCROSE TOLERANCE TESTS IN DIABETES MELLITUS

| CASE | HOSPITAL NUMBER | AGE | SEX | GLUCOSE TOLERANCE TESTS | | | | SUCROSE TOLERANCE TESTS | | | | DIAGNOSIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | DATE | TIME HR. | BLOOD SUGAR MG. PER 100 C.C. | URINE SUGAR | DATE | TIME HR. | BLOOD SUGAR MG. PER 100 C.C. | URINE SUGAR | |
| 19 | Out | 43 | M | 3/23/33 | F. | 167 | + | 4/ 1/33 | F. | 179 | + | Diabetes mellitus |
| | | | | | 1 | 435 | ++++ | | 1 | 333 | ++ | |
| | | | | | 2 | 480 | ++++ | | 2 | 333 | ++++ | |
| | | | | | 3 | 367 | ++++ | | 3 | 250 | ++++ | |
| | | | | | | | (14.3 gm.) | | | | (14.7 gm.) | |
| 20 | 8858 | 46 | M | 1/ 6/33 | F. | 114 | Neg. | 1/19/33 | F. | 110 | Neg. | Diabetes mellitus, emphysema, chronic bronchitis, arthritic spine (early) |
| | | | | | 1 | 308 | Tr. | | 1 | 250 | +++ | |
| | | | | | 2 | 286 | ++++ | | 2 | 182 | +++ | |
| | | | | | 3 | 233 | ++++ | | 3 | 154 | +++ | |
| 21 | 9114 | 54 | M | 4/24/33 | F. | 137 | Neg. | 4/28/33 | F. | 130 | Neg. | Diabetes mellitus, arteriosclerotic gangrene foot, caries, chronic bronchitis |
| | | | | | 1 | 250 | Neg. | | 1 | 250 | Neg. | |
| | | | | | 2 | 282 | ++ | | 2 | 263 | + | |
| | | | | | 3 | 277 | ++++ | | 3 | 222 | ++ | |
| | | | | | | | (1.56 gm.) | 4/28/33 | | | (0.64 gm.) | |
| 22 | 3856 | 42 | F | 10/29/31 | F. | 100 | Tr. | 11/ 4/31 | F. | 118 | Neg. | Diabetes mellitus, arteriosclerotic cardiovascular disease |
| | | | | | 1 | 286 | ++++ | | 1 | 235 | ++++ | |
| | | | | | 2 | 190 | ++++ | | 2 | 159 | ++ | |
| | | | | | 3 | 95 | ++++ | | 3 | 99 | Neg. | |
| 23 | 7874 | 70 | M | 10/29/32 | F. | 125 | Neg. | 11/ 5/32 | F. | 118 | Neg. | Diabetes mellitus, bilateral cataracts, fracture upper right tibia |
| | | | | | 1 | 211 | Neg. | | 1 | 238 | Neg. | |
| | | | | | 2 | 200 | + | | 2 | 222 | + | |
| | | | | | 3 | 198 | + | | 3 | 187 | + | |
| 24 | 14673 | 55 | F | 7/13/34 | F. | 109 | Neg. | 7/24/34 | F. | 123 | Neg. | Diabetes mellitus, obesity, coronary thrombosis, arteriosclerosis |
| | | | | | 1 | 235 | Neg. | | 1 | 233 | Neg. | |
| | | | | | 2 | 270 | + | | 2 | 256 | + | |
| | | | | | 3 | 286 | Tr. | | 3 | 244 | Tr. | |

TABLE 1.—CONT'D

| CASE | HOSPITAL NUMBER | AGE | SEX | GLUCOSE TOLERANCE TESTS | | | | SUCROSE TOLERANCE TESTS | | | | DIAGNOSIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | DATE | TIME HR. | BLOOD SUGAR MG. PER 100 C.C. | URINE SUGAR | DATE | TIME HR. | BLOOD SUGAR MG. PER 100 C.C. | URINE SUGAR | |
| 25 | 15045 | 50 | F | 7/26/34 | F. | 114 | Neg. | 7/21/34 | F. | 106 | Neg. | Diabetes mellitus, menopause |
| | | | | | 1 | 222 | Neg. | | 1 | 225 | + | |
| | | | | | 2 | 169 | + | | 2 | 138 | Neg. | |
| | | | | | 3 | 114 | Neg. | | 3 | 110 | Neg. | |
| 26 | 14104 | 35 | M | 5/14/34 | F. | 111 | Neg. | 5/ 7/34 | F. | 100 | Neg. | Diabetes mellitus, cerebral angiospasm, pterygium right eye |
| | | | | | 1 | 235 | ++ | | 1 | 167 | + | |
| | | | | | 2 | 133 | +++ | | 2 | 103 | + | |
| | | | | | 3 | 110 | Neg. | | 3 | 103 | Neg. | |
| 27 | 9799 | 45 | M | 4/ 8/33 | F. | 118 | Neg. | 4/13/33 | F. | 118 | Neg. | Diabetes mellitus, infected teeth, dental caries |
| | | | | | 1 | 175 | Neg. | | 1 | 202 | + | |
| | | | | | 2 | 182 | + | | 2 | 205 | ++ | |
| | | | | | 3 | 145 | + | | 3 | 134 | ++++ | |
| 28 | Out | 25 | M | 11/ 5/32 | F. | 105 | Neg. | 3/ 4/33 | F. | 105 | Neg. | Potential diabetes |
| | | | | | 1 | 178 | ++++ | | 1 | 154 | ++ | |
| | | | | | 2 | 121 | Tr. | | 2 | 118 | + | |
| | | | | | 3 | 119 | Neg. | | 3 | 111 | Tr. | |
| 29 | 9342 | 46 | M | 2/25/33 | F. | 95 | Neg. | 2/28/33 | F. | 98 | Neg. | Diabetes mellitus, detachment of tenth and eleventh costal cartilage |
| | | | | | 1 | 227 | Neg. | | 1 | 206 | Neg. | |
| | | | | | 2 | 241 | + | | 2 | 142 | + | |
| | | | | | 3 | 165 | + | | 3 | 100 | Neg. | |
| 30 | Out | 40 | M | 6/ 4/34 | F. | 118 | Neg. | 6/ 7/34 | F. | 111 | Neg. | Diabetes mellitus |
| | | | | | 1 | 211 | + | | 1 | 211 | Neg. | |
| | | | | | 2 | 117 | ++ | | 2 | 87 | ++ | |
| | | | | | 3 | 111 | Neg. (0.34 gm.) | | 3 | 81 | Tr. (0.40 gm.) | |

eign body, unchanged. While the above experiment was performed with ordinary fasting blood it does not seem probable, in view of the work of Abderhalden and Buadze (1932), that the ingestion of cane sugar could effect

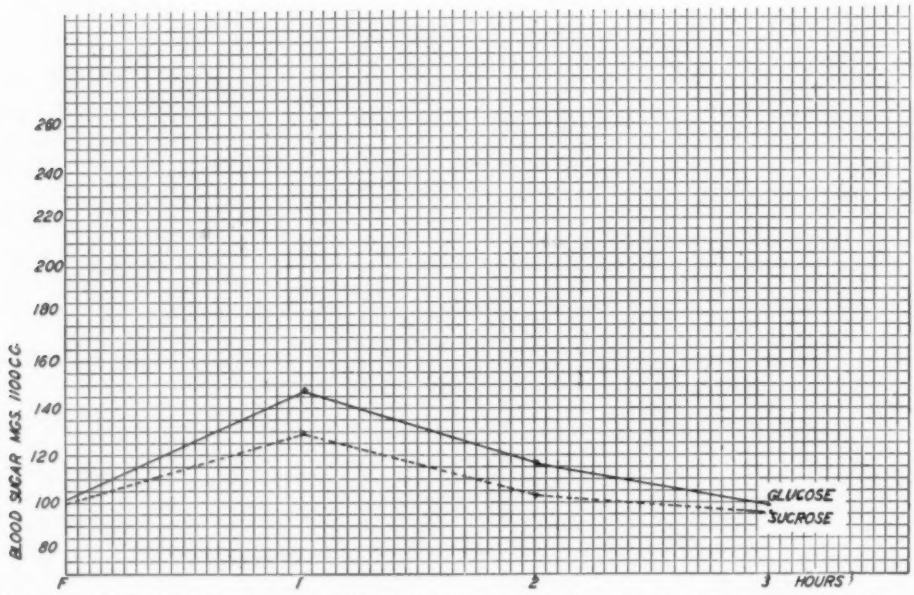


Chart 1.—Normal blood sugar curves following the ingestion of glucose or sucrose (1.5 gm. per kilo). Average of 18 separate cases.

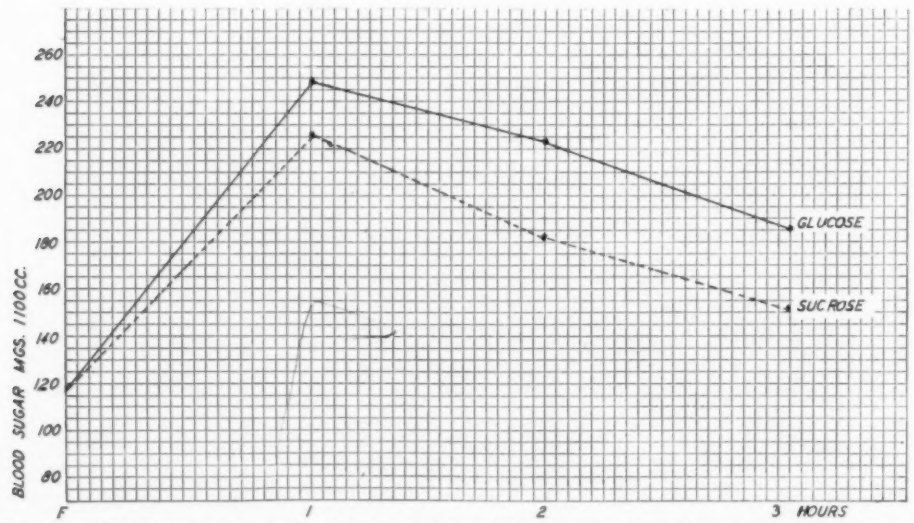


Chart 2.—Blood sugar curves in diabetes mellitus following the ingestion of glucose or sucrose (1.5 gm. per kilo). Average of 12 separate cases.

the mobilization of appreciable quantities of sucrase into the blood stream within a few minutes.

## RESULTS

During the course of this investigation glucose and sucrose tolerance tests have been carried out upon 57 separate hospital patients. No attempt

has been made to secure a series of normal controls, since we feel that other workers have amply demonstrated the fact that healthy individuals, upon the ingestion of cane sugar, yield blood sugar time curves well within the normal range.

The data have been collected into three groups. The first group (Chart 1) contains the summarized data on eighteen cases which, although pathologic in nature, yielded relatively normal blood sugar curves with both glucose and sucrose. Table I and Chart 2 contain the experimental findings on twelve cases of diabetes mellitus. With one exception (No. 19) these patients were admitted for conditions other than diabetes and only through tolerance studies were the diabetic tendencies discovered. Due to the disturbing influence of large quantities of sugar we have hesitated to subject known diabetic patients to these tests. The third group of patients (Table II and Chart 3) includes twenty-seven cases involving infectious or arthritic conditions of

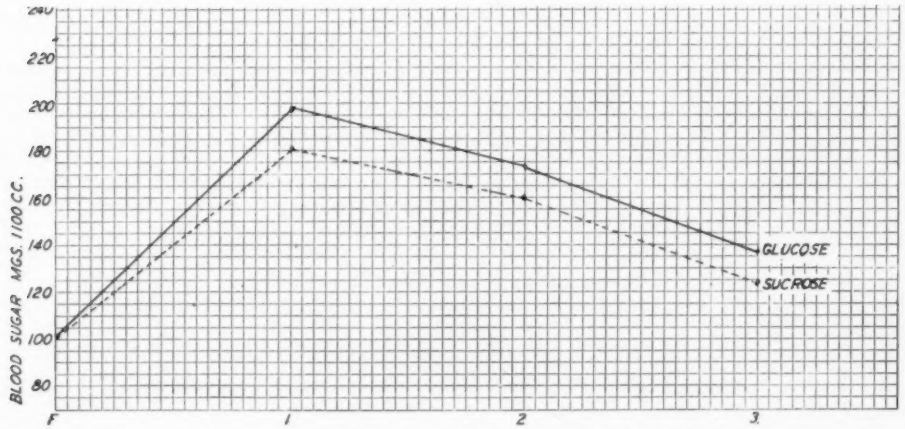


Chart 3.—Blood sugar curves in infections, arthritis, etc., following the ingestion of glucose or sucrose (1.5 gm. per kilo). Average of 27 separate cases.

various types which, in general, yielded abnormally high blood sugar time curves with both sugars.

In studying the data given by these two tolerance tests we were interested primarily in discovering whether both sugars give the same type of blood and urine sugar curves. In short, will the ingestion of cane sugar yield the same clinical information as a like quantity of glucose?

## COMMENT AND SUMMARY

1. Fifty-seven hospital cases have been subjected to both glucose and sucrose tolerance tests and the respective blood and urine sugar curves studied comparatively. While individual differences were found between the various blood sugar curves, their general pattern was similar. A group of eighteen patients (Chart 1), free from obvious carbohydrate metabolism abnormalities, gave normal blood sugar curves and urines negative to Benedict's solution with both sugars. The average blood sugar values following sucrose ingestion were 100.7, 129.2, 102.3, and 95.5 mg. per cent for the fast-

TABLE II

COMPARISON OF GLUCOSE AND SUCROSE TOLERANCE TESTS IN INFECTIONS AND ALLIED CONDITIONS

| CASE | HOSPITAL NUMBER | AGE | SEX | GLUCOSE TOLERANCE TESTS | | | | | SUCROSE TOLERANCE TESTS | | | | | DIAGNOSIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | DATE | TEMP. °F. | TIME HR. | BLOOD SUGAR MG. PER 100 C.C. | URINE SUGAR | DATE | TEMP. °F. | TIME HR. | BLOOD SUGAR MG. PER 100 C.C. | URINE SUGAR | |
| 31 | 8438 | 55 | M | 12/ 3/32 | 98.6 | F. | 127 | Neg. | 11/30/32 | 98.6 | F. | 100 | Neg. | Hypertensive cardiovascular disease, tertiary syphilis, bronchitis, left hydrocele |
| | | | | | | 1 | 286 | Neg. | | | 1 | 200 | Neg. | |
| | | | | | | 2 | 253 | Neg. | | | 2 | 204 | Neg. | |
| | | | | | | 3 | 197 | Neg. | | | 3 | 147 | Neg. | |
| 32 | 8311 | 54 | F | 11/21/31 | 100.0 | F. | 125 | Neg. | 11/21/32 | 100.0 | F. | 114 | Neg. | Infectious arthritis, caries pyorrhea, chronic tonsillitis |
| | | | | | | 1 | 250 | Neg. | | | 1 | 222 | Neg. | |
| | | | | | | 2 | 250 | + | | | 2 | 201 | Neg. | |
| | | | | | | 3 | 199 | Tr. | | | 3 | 116 | Neg. | |
| 33 | 7683 | 25 | M | 10/ 3/32 | 98.6 | F. | 100 | Neg. | 10/ 6/32 | 98.6 | F. | 105 | Neg. | Syphilis, secondary anemia, agranulocytic anemia (convalescent period) |
| | | | | | | 1 | 211 | Neg. | | | 1 | 230 | Neg. | |
| | | | | | | 2 | 175 | Neg. | | | 2 | 161 | Neg. | |
| | | | | | | 3 | 111 | Neg. | | | 3 | 95 | Neg. | |
| 34 | 6407 | 71 | M | 6/ 9/32 | 100.2 | F. | 98 | Neg. | 6/13/32 | 99.2 | F. | 100 | Neg. | Tenosynovitis right shoulder, purulent otitis media |
| | | | | | | 1 | 142 | Neg. | | | 1 | 167 | Neg. | |
| | | | | | | 2 | 217 | Neg. | | | 2 | 208 | Neg. | |
| | | | | | | 3 | 204 | | | | 3 | 184 | Neg. | |
| 35 | 6362 | 70 | M | 6/ 7/32 | 98.2 | F. | 91 | Neg. | 6/13/32 | 98.6 | F. | 91 | Neg. | Multiple infectious arthritis, chronic infected tonsils |
| | | | | | | 1 | 241 | Neg. | | | 1 | 200 | Neg. | |
| | | | | | | 2 | 217 | ++++ | | | 2 | 192 | Neg. | |
| | | | | | | 3 | 190 | ++++ | | | 3 | 167 | Neg. | |
| 36 | 5796 | 40 | F | 4/23/32 | | F. | 95 | Neg. | 4/29/32 | | F. | 93 | Neg. | Infectious arthritis, tonsillitis, pyorrhea, alveolaris |
| | | | | | | 1 | 190 | Neg. | | | 1 | 134 | Neg. | |
| | | | | | | 2 | 152 | Neg. | | | 2 | 125 | Neg. | |
| | | | | | | 3 | 128 | Neg. | | | 3 | 88 | Neg. | |
| 37 | 8396 | 53 | M | 12/21/32 | 98.6 | F. | 91 | Neg. | 11/30/32 | 98.6 | F. | 100 | Neg. | Hallux valgus deformity left foot, lymphangitis right knee |
| | | | | | | 1 | 153 | Neg. | | | 1 | 157 | Neg. | |
| | | | | | | 2 | 125 | Neg. | | | 2 | 145 | Neg. | |
| | | | | | | 3 | 116 | Neg. | | | 3 | 133 | Neg. | |

TABLE II—CONT'D

| CASE | HOSPITAL NUMBER | AGE | SEX | GLUCOSE TOLERANCE TESTS | | | | | SUCROSE TOLERANCE TESTS | | | | | DIAGNOSIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | DATE | TEMP. °F. | TIME HR. | BLOOD SUGAR MG. PER 100 C.C. | URINE SUGAR | DATE | TEMP. °F. | TIME HR. | BLOOD SUGAR MG. PER 100 C.C. | URINE SUGAR | |
| 38 | 4270 | 52 | M | 1/11/32 | 99.4 | F. | 95 | Neg. | 1/ 7/32 | 99.6 | F. | 91 | Neg. | Acute pulmonary tuberculosis, pleurisy |
| | | | | | | 1 | 187 | Neg. | | | 1 | 222 | Neg. | |
| | | | | | | 2 | 174 | Neg. | | | 2 | 142 | Neg. | |
| | | | | | | 3 | 87 | Neg. | | | 3 | 80 | Neg. | |
| 39 | 13932 | 55 | F | 4/12/34 | 98.8 | F. | 94 | Neg. | 4/14/34 | 99.0 | F. | 108 | Neg. | Hypertrophic arthritis right knee, syphilis, hypertensive cardiovascular disease |
| | | | | | | 1 | 238 | Neg. | | | 1 | 235 | Neg. | |
| | | | | | | 2 | 227 | Neg. | | | 2 | 167 | Neg. | |
| | | | | | | 3 | 179 | Neg. | | | 3 | 145 | Neg. | |
| 40 | 9071 | 42 | M | 2/ 4/33 | 99.8 | F. | 100 | Neg. | 2/ 2/33 | 99.4 | F. | 102 | Neg. | Rheumatic cardiovascular disease with aortic and mitral insufficiency, subacute bacterial endocarditis, syphilis, hemiplegia |
| | | | | | | 1 | 170 | Neg. | | | 1 | 190 | Neg. | |
| | | | | | | 2 | 155 | Neg. | | | 2 | 138 | Neg. | |
| | | | | | | 3 | 125 | Neg. | | | 3 | 98 | Neg. | |
| 41 | 9886 | 48 | M | 4/24/33 | 100.0 | F. | 100 | Neg. | 4/28/33 | 101.4 | F. | 100 | Neg. | Pulmonary abscess, dental caries, pyorrhea |
| | | | | | | 1 | 125 | Neg. | | | 1 | 142 | Neg. | |
| | | | | | | 2 | 123 | Neg. | | | 2 | 118 | Neg. | |
| | | | | | | 3 | 110 | Neg. | | | 3 | 114 | Neg. | |
| 42 | 9433 | 58 | F | 3/ 7/33 | 98.9 | F. | 106 | Neg. | 3/11/33 | 98.9 | F. | 106 | Neg. | Active pulmonary tuberculosis, paroxysmal hypertension, mitral stenosis |
| | | | | | | 1 | 260 | Tr. | | | 1 | 167 | Tr. | |
| | | | | | | 2 | 159 | + | | | 2 | 200 | ++++ | |
| | | | | | | 3 | 122 | ++ | | | 3 | 128 | ++ | |
| 43 | 13166 | 41 | M | 2/ 9/34 | 98.6 | F. | 99 | Neg. | 2/12/34 | 99.2 | F. | 97 | Neg. | Toxic polyneuritis, hyperkeratosis of feet |
| | | | | | | 1 | 206 | Neg. | | | 1 | 163 | Neg. | |
| | | | | | | 2 | 133 | Neg. | | | 2 | 182 | Neg. | |
| | | | | | | 3 | 109 | Neg. | | | 3 | 142 | Neg. | |
| 44 | 4312 | 35 | F | 1/29/32 | 98.6 | F. | 111 | Neg. | 1/26/32 | 98.6 | F. | 105 | Neg. | Syphilis, typhoid fever (late convalescent period) |
| | | | | | | 1 | 230 | Neg. | | | 1 | 190 | Neg. | |
| | | | | | | 2 | 160 | Neg. | | | 2 | 133 | Neg. | |
| | | | | | | 3 | 80 | Neg. | | | 3 | 91 | Neg. | |

TABLE II—CONT'D

| CASE | HOSPITAL NUMBER | AGE | SEX | GLUCOSE TOLERANCE TESTS | | | | | SUCROSE TOLERANCE TESTS | | | | | DIAGNOSIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | DATE | TEMP. °F. | TIME HR. | BLOOD SUGAR MG. PER 100 C.C. | URINE SUGAR | DATE | TEMP. °F. | TIME HR. | BLOOD SUGAR MG. PER 100 C.C. | URINE SUGAR | |
| 45 | 9435 | 67 | M | 3/ 6/33 | 98.6 | F. | 111 | Neg. | 3/ 9/33 | 98.6 | F. | 109 | Neg. | Gout, generalized arteriosclerosis, coronary sclerosis |
| | | | | | | 1 | 161 | Neg. | | | 1 | 211 | Neg. | |
| | | | | | | 2 | 125 | Neg. | | | 2 | 200 | Neg. | |
| | | | | | | 3 | 114 | Neg. | | | 3 | 154 | Neg. | |
| 46 | 9312 | 29 | M | 3/ 9/33 | 99.8 | F. | 100 | Neg. | 2/28/33 | 99.8 | F. | 105 | Neg. | Syphilitic cardiovascular disease, aneurism arch aorta, occlusion left main bronchus |
| | | | | | | 1 | 200 | Neg. | | | 1 | 167 | Neg. | |
| | | | | | | 2 | 164 | Neg. | | | 2 | 137 | Neg. | |
| | | | | | | 3 | 111 | Neg. | | | 3 | 110 | Neg. | |
| 47 | 7781 | 62 | M | 9/24/32 | 100.0 | F. | 128 | Neg. | 9/23/32 | 100.8 | F. | 160 | Neg. | Cellulitis left leg |
| | | | | | | 1 | 200 | Neg. | | | 1 | 308 | Neg. | |
| | | | | | | 2 | 213 | Tr. | | | 2 | 333 | Tr. | |
| | | | | | | 3 | 198 | Neg. | | | 3 | 303 | Neg. | |
| 48 | 7781 | 62 | M | 10/21/32 | 98.6 | F. | 114 | Neg. | 10/18/32 | 98.6 | F. | 115 | Neg. | Cellulitis left leg (recovery practically complete) |
| | | | | | | 1 | 194 | Neg. | | | 1 | 179 | Neg. | |
| | | | | | | 2 | 160 | Neg. | | | 2 | 114 | Neg. | |
| | | | | | | 3 | 108 | Neg. | | | 3 | 95 | Neg. | |
| 49 | 3693 | 25 | M | 4/ 5/34 | 100.0 | F. | 108 | Neg. | 4/ 9/34 | 98.6 | F. | 88 | Neg. | Recurrent lymphangitis leg, infected tonsils, acute infectious arthritis, (some improvement when sucrose test was performed) |
| | | | | | | 1 | 154 | Neg. | | | 1 | 95 | Tr. | |
| | | | | | | 2 | 127 | Neg. | | | 2 | 96 | Tr. | |
| | | | | | | 3 | 121 | Neg. | | | 3 | 91 | Tr. | |
| 50 | 13063 | 46 | M | 2/ 7/34 | 99.6 | F. | 83 | Neg. | 2/ 9/34 | 100.2 | F. | 108 | Neg. | Cerebral vascular syphilis with hemiplegia. |
| | | | | | | 1 | 154 | Neg. | | | 1 | 135 | Neg. | |
| | | | | | | 2 | 150 | Neg. | | | 2 | 156 | Neg. | |
| | | | | | | 3 | 133 | Neg. | | | 3 | 105 | Neg. | |
| 51 | 7741 | 40 | M | 2/ 5/33 | 99.0 | F. | 100 | Neg. | 2/ 2/33 | 99.0 | F. | 102 | Neg. | Chronic arthritis |
| | | | | | | 1 | 170 | Neg. | | | 1 | 190 | Neg. | |
| | | | | | | 2 | 155 | Neg. | | | 2 | 138 | Neg. | |
| | | | | | | 3 | 125 | Neg. | | | 3 | 98 | Neg. | |

TABLE II—CONT'D

| CASE | HOSPITAL NUMBER | AGE | SEX | GLUCOSE TOLERANCE TESTS | | | | | SUCROSE TOLERANCE TESTS | | | | | DIAGNOSIS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | DATE | TEMP. °F. | TIME HR. | BLOOD SUGAR MG. PER 100 C.C. | URINE SUGAR | DATE | TEMP. °F. | TIME HR. | BLOOD SUGAR MG. PER 100 C.C. | URINE SUGAR | |
| 52 | 14243 | 41 | M | 5/12/34 | 100.0 | F. | 95 | Neg. | 5/15/34 | 99.2 | F. | 100 | Neg. | Chronic multiple arthritis, pleurisy with effusion, infected tonsils |
| | | | | | | 1 | 185 | Neg. | | | 1 | 133 | Neg. | |
| | | | | | | 2 | 136 | Neg. | | | 2 | 133 | Neg. | |
| | | | | | | 3 | 116 | Neg. | | | 3 | 130 | Neg. | |
| 53 | 13722 | 42 | F | 4/29/34 | 99.0 | F. | 114 | Neg. | 5/ 5/34 | 98.7 | F. | 100 | Neg. | Chronic multiple arthritis, colloid and adenomatous goiter, dental caries |
| | | | | | | 1 | 179 | Neg. | | | 1 | 175 | Neg. | |
| | | | | | | 2 | 204 | + | | | 2 | 142 | Neg. | |
| | | | | | | 3 | 133 | Neg. | | | 3 | 136 | Neg. | |
| 54 | 9446 | 34 | M | 3/ 6/33 | 98.6 | F. | 100 | Neg. | 3/14/33 | 98.6 | F. | 100 | Neg. | Acute rheumatic fever, chronic infected tonsils, chronic sinusitis |
| | | | | | | 1 | 136 | Neg. | | | 1 | 160 | Neg. | |
| | | | | | | 2 | 118 | Neg. | | | 2 | 135 | Neg. | |
| | | | | | | 3 | 91 | Neg. | | | 3 | 87 | Neg. | |
| 55 | 14029 | 51 | M | 4/23/34 | 100.0 | F. | 108 | Neg. | 4/27/34 | 100.2 | F. | 105 | Neg. | Acute infectious arthritis, chronic prostatitis, obesity, dental caries |
| | | | | | | 1 | 250 | Neg. | | | 1 | 204 | Neg. | |
| | | | | | | 2 | 222 | ++ | | | 2 | 129 | + | |
| | | | | | | 3 | 174 | Tr. | | | 3 | 118 | Neg. | |
| 56 | 14051 | 35 | F | 4/26/34 | 99.0 | F. | 90 | Neg. | 5/14/34 | 99.0 | F. | 98 | Neg. | Hypertensive cardiovascular disease, old hemiplegia |
| | | | | | | 1 | 190 | Neg. | | | 1 | 160 | Neg. | |
| | | | | | | 2 | 200 | + | | | 2 | 136 | Neg. | |
| | | | | | | 3 | 167 | Neg. | | | 3 | 105 | Neg. | |
| 57 | 14930 | 42 | F | 7/21/34 | 99.0 | F. | 118 | Neg. | 5/19/34 | 99.0 | F. | 104 | Neg. | Abdominal adhesions, diastasis recti |
| | | | | | | 1 | 194 | Neg. | | | 1 | 189 | Neg. | |
| | | | | | | 2 | 200 | Neg. | | | 2 | 167 | Neg. | |
| | | | | | | 3 | 148 | Neg. | | | 3 | 80 | Neg. | |

ing, one-hour, two-hour, and three-hour periods, respectively. These same patients gave, upon the administration of a similar amount of glucose (1.5 gm. per kilogram of body weight), blood sugar values of 101.4, 146.0, 117.2, and 98.2 per cent at the same time intervals. Thus the average blood sugar curve given by the sucrose meal was 11.51, 12.71, and 2.75 per cent lower than that given by glucose for the one-hour, two-hour, and three-hour periods, respectively. It is evident, therefore, that the ingestion of approximately 100 gm. of sucrose ordinarily yields a blood sugar curve well within the normal limits as established by glucose tolerance tests. The urines were free from sucrose or reducing sugars regardless of the type of sugar ingested.

2. Twelve patients with diabetes mellitus showed marked hyperglycemia and glycosuria with both sugars (Table I and Chart 2). The highest blood sugar value (Case 19) given by sucrose was 333 mg. per cent as compared to 480 mg. per cent given by the same patient when glucose was administered. The average blood sugar values for these sucrose tolerance tests were 118.0, 225.3, 184.0, and 149.6 mg. per cent for the fasting, one-hour, two-hour, and three-hour periods, respectively, as compared to 117.8, 247.8, 222.6, and 185.0 mg. per cent for glucose at the respective time intervals. Thus the blood sugar response to sucrose ingestion was 9.8, 17.34, and 19.13 per cent lower than that given by glucose for the one-, two- and three-hour intervals, respectively. Both sugars resulted in glycosuria to approximately the same extent. In no case did the administration of sucrose fail to bring out the diabetic tendencies of these patients. Hence it is our opinion that clinically sucrose is as satisfactory as glucose for the detection and evaluation of diabetes mellitus.

3. Twenty-seven patients with various arthritic and infectious conditions have also been studied in a like manner (Table II and Chart 3). The average blood sugar values following sucrose ingestion were 103.9, 183.4, 160.4, and 123.7 mg. per cent for the fasting, one-hour, two-hour, and three-hour periods, respectively, as compared to 103.7, 198.3, 173.8, and 136.9 mg. per cent for glucose at similar time intervals. Thus these patients showed a blood sugar response to sucrose ingestion 8.02, 7.71, and 9.64 per cent lower than the response given by glucose at the various periods of the test. However, one can readily conclude from the data that sucrose tolerance tests show as well as glucose tolerance tests that an abnormality in carbohydrate metabolism is present during arthritis, infections, and similar conditions. In addition, sucrose ingestion shows equally well that this abnormality disappears as the infection clears up. Material differences between consecutive blood sugar curves following ingestion of sugar need not always be surprising since recovery from the infection rapidly increases the patient's ability to remove sugar from the blood. It seems obvious that in some of the cases where material differences between the curves were noted, an improvement, or otherwise, in the condition of the patient could have taken place without being evident clinically. Case 47-48 is interesting in this regard. The data secured

on this patient clearly proves that sucrose tolerance tests bring out the improvement in carbohydrate tolerance as well as does glucose when the infection has cleared up.

As a rule, despite the high values for the blood sugars, the urines were usually free from reducing sugars. Glycosuria was present during six glucose tolerance tests and during two sucrose tolerance tests. Case 35 differed materially in that glucose ingestion resulted in 4-plus urine sugar at the second and third hourly periods, whereas the urine was free from sugar following the sucrose meal. The blood sugar curves were abnormally high with both sugars, however. Whether glucose intake results in more frequent glycosuria than does sucrose can only be proved by more extensive studies.

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# THE BACTERICIDAL AND PHOTOCHEMICAL PROPERTIES OF IRRADIATED PETROLATUM AND MINERAL OIL*

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IN 1931 Eising[1] suggested the use of irradiated petrolatum for the treatment of infected wounds and sinuses. He had observed that with this treatment the bacterial count in the discharge from the wounds diminished, the growth of granulation tissue was stimulated and healing was hastened. These effects were attributed to a bactericidal action together with a stimulus to healing, both of which he believed were due to secondary rays emanating from petrolatum following ultraviolet irradiation. He found that photographic plates could be sensitized to subsequent development if they were placed in the dark over petrolatum previously exposed to ultraviolet light from which the longer wave lengths had been filtered. Exposure occurred even though the plates were sealed between sheets of x-ray film from which the emulsion had been removed. From these observations he concluded that the therapeutic value of the oil was due to secondary radiant energy dependent on exposure to ultraviolet light rays. In subsequent articles[2, 3] he attributed the photochemical action of the irradiated petrolatum to heavy gaseous reducing agents and not to actinism. Even though infection apparently subsided in wounds which were treated, the petrolatum failed to kill bacteria in vitro. He remarks, however, that if a loop of *Staphylococcus aureus* from an agar slant is suspended finely in irradiated oil, according to the method of Thompson and Sheard,[4] subcultures from this oil show no growth after eight hours. He concludes that the bactericidal action differs from that of ordinary germicides in that it is slow, that the vapor affecting the photographic plate is not the same as the bactericidal vapor arising from some irradiated vegetable and animal oils, and that the factor which promotes healing may be distinct from that detrimental to the growth of bacteria.

While it has been impractical to carry out extensive laboratory experiments on the growth-stimulating qualities of irradiated petrolatum, the photochemical and bactericidal properties have been investigated. The effects on both aerobic and anaerobic bacteria have been studied. Unfortunately, so little if any bactericidal effect has been observed, that the correlation between the bactericidal and photosensitizing qualities could not be investigated.

## PHOTOCHEMICAL EFFECTS

The first few experiments confirmed some but not all of the observations previously recorded in the literature. Normal petrolatum failed to fog photographic plates, but irradiated petrolatum did fog them within twenty-four hours.

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Glass or quartz placed between the surface of the petrolatum and the emulsion prevented fogging, but the interposition of a film of acetocellulose failed to abolish the effect. In these and all subsequent exposures, the petrolatum was irradiated under a quartz-mercury arc at 30 cm. in flat dishes with uniform surface exposures. The photochemical effects were tested with 10 c.c. of irradiated oil in Stender jars of uniform size. Eastman hypersensitive press plates were inverted over these jars for twenty-four hours.

After confirmation of the observation that plates were affected through washed x-ray film but not through glass or quartz, the assumption that the fogging effect resulted from vapor from the irradiated petrolatum was subjected to experimental study. A Stender jar was partially filled with irradiated petrolatum, a plate was sealed to the rim with gelatin, and a current of air was drawn rapidly over the surface of the petrolatum through an inlet and outlet sealed in opposite sides of the jar. The plate was unfogged after twenty-four hours. Both normal and irradiated petrolatum were then alternately degassed in a vacuum and saturated with carbon dioxide, nitrogen, or oxygen. If petrolatum previously activated by ultraviolet light was washed successively with any one of these gases, samples removed after each washing showed a steady decrease in the photochemical effect. Six washings usually abolished the effect entirely. Normal oil similarly degassed and washed repeatedly with nitrogen or carbon dioxide was not activated by ultraviolet light after several washings, even if finally saturated with oxygen before irradiation. If oxygen was used for washing, samples removed after each degassing and irradiated showed a less rapid decrease in photochemical effect, when compared with similar samples washed with carbon dioxide and saturated once with oxygen. More washings were required with oxygen than with carbon dioxide to abolish completely the fogging effect. A current of air bubbled through irradiated mineral oil partially removed the substance responsible for fogging. On the assumption that this substance was vaporized, air was bubbled through several hundred cubic centimeters of irradiated mineral oil, collected as it escaped from the oil, conducted through a 1 mm. quartz tube lying against the emulsion of a photographic plate, and then allowed to escape from a capillary tube against the surface of a film of acetocellulose pressed firmly against a second plate. The first plate was unexposed where the tube lay against it; the second was fogged where the air current flowed against the film of acetocellulose. These experiments seem to show conclusively that the fogging effects obtained with irradiated petrolatum are caused by a vapor which is readily diffusible and must come in contact with the emulsion to affect the plate.

In one of the previous experiments, oil fully saturated with oxygen previous to irradiation appeared more photoactive than a sample less fully saturated. This experiment suggested that oxygen might be necessary for the activation of the inactive substance. An ideal experiment was impossible. Manipulation designed to completely remove the oxygen prior to irradiation removed the precursor of the substance responsible for fogging as well. As a compromise, the effects of practically equivalent saturation with oxygen and nitrogen prior to radiation were compared. A sample of normal oil was degassed and satu-

rated twice with carbon dioxide. This reduced the fogging effect on plates to a point where comparisons could be readily made. Equal volumes of the oil, still saturated with carbon dioxide, were transferred to two identical vessels. The vessels were covered with clear cellophane. Equal volumes of nitrogen and oxygen were then bubbled through these samples at equal rates while the samples were exposed similarly to ultraviolet light. The sample treated with nitrogen was photochemically inactive but intense activity was obtained with the oxygen-treated sample, proving the necessity for oxygen in the process of activation.

With the well-recognized oxidizing effect of ultraviolet light in mind, the dependence on oxygen for the photochemical activation of the oil suggested that active organic peroxides might be formed. Harris, Bunker, and Milas[5, 6] have found peroxides in the vapor above irradiated oils. Tests for peroxidic substances immediately above the surface of irradiated petrolatum confirmed their observations. They found no aldehydes, nor did they obtain diffusion of peroxides through x-ray film. Technical difficulties have rendered tests for aldehydes in the vapor immediately above active oils inconclusive. Experiments have shown, however, that the peroxides diffuse through a film of acetocellulose and that aldehydes are to be found with them. Sections of washed x-ray film were coated with pure gelatin. When the coatings were nearly dry the covers of Petri dishes were pressed rim down on the films of gelatin. The covers with the adhering sections of x-ray film were then placed over the bottoms of the dishes which were partially filled with petrolatum. Some of the gelatin films were plain, others impregnated with starch. Some dishes were filled with normal and others with irradiated petrolatum. After several hours at 38° C., the sections of film were removed and the gelatin scraped off each into tubes of cold water. A blue color was developed in the tubes of the starch emulsion from the dishes of irradiated petrolatum when an acidified iodide solution was added. The scrapings of plain gelatin were red after Schiff's reagent had been added to these tubes. The control reactions with gelatin exposed over normal petrolatum were negative with both reagents. The Schiff reagent used gave negative reactions with perhydrol.

The previous experiments permit the conclusion that the photoactivity of irradiated petrolatum and mineral oil is chemical, that a substance is activated by ultraviolet light in the presence of oxygen which vaporizes readily, that organic peroxides and aldehydes are formed, and that these aldehydes and peroxides diffuse readily through a film of acetocellulose.

## BACTERICIDAL EFFECTS

Although many essential oils are normally bactericidal, an effect occurring chiefly with those containing alcohols or aldehydes, the effect of the vapors has been little studied. Experiments in this direction are difficult because vaporization occurs so readily that bacteria exposed to the vapor are soon smothered in a coating of oil. Hence, a comparison of the bactericidal effects, before and after irradiation, of the oils or the vapors of oils which normally kill bacteria is unsatisfactorily inconclusive. But Wrenn,[7] in 1927, observed bactericidal action with vapors of irradiated oils which were not bactericides in the un-

irradiated state. With inconclusive evidence he attributed this activity to light emanations. Harris, Bunker and Milas, just recently, after an extensive study of the bactericidal effects of vaporous emanations from numerous irradiated vegetable and animal oils, concluded that the bactericidal effects were due to peroxides. They found a direct correlation, not between the initial or final peroxidic oxygen content of irradiated oils, but between the increase in peroxidic oxygen content occurring during irradiation, and the germicidal effect of their vapors.

Investigations of the bactericidal effects of irradiated petrolatum and mineral oil have been made. Ross[8] reported that *Staphylococcus aureus*, but not *Bacillus pyocyaneus*, was killed when a loop of these bacteria from agar was emulsified in an irradiated mixture of petrolatum and lanolin. Thompson and Sheard repeated these experiments. They made bactericidal counts from hour to hour from the emulsion of bacteria and irradiated petrolatum and compared these counts with counts of bacteria suspended in normal petrolatum. The counts on the irradiated mixture showed a steady and rapid decrease in bacteria. A decrease was also encountered in the unirradiated mixture. Harris, Bunker and Milas exposed films of bacteria on agar plates to the vapor over irradiated mineral oil. The bacteria were not killed. These in vitro experiments, however, fail to furnish the conditions under which a clinical germicide must act. Sears and Black[9] have recently carried out in vitro experiments, simulating the conditions under which a bactericidal oil must act in vivo. The anaerobes studied were not affected, nor were bacilli of the colon-typhoid group killed by irradiated petrolatum. Staphylococcus and streptococcus, both viridans and hemolyticus, were not viable after prolonged contact with the irradiated oil. These authors conclude that the mild bactericidal effect observed is due to a nonvolatile compound formed under the oxidative action of ultraviolet light.

To exactly simulate in vitro the conditions encountered in an infected wound is impossible. The nearest approach is an experiment in which the bacteria are suspended in minute globules of nutrient media emulsified in the oil or petrolatum. In preliminary experiments freshly spread films of several aerobic pathogens on blood agar plates were exposed twenty-four hours in the incubator to the vapor over irradiated petrolatum. Several common anaerobes were similarly exposed during incubation in Fildes jars. Next, 1 c.c. of liquid culture of each of these bacteria was centrifuged, and the residual bacterial sediment thoroughly mixed with 5 c.c. of warmed irradiated petrolatum. Cultures were made in twenty-four hours. In the third experiment 1 c.c. of uncentrifuged liquid culture of each of these bacteria was emulsified in irradiated mixtures of mineral oil and petrolatum of a consistency in which a fine suspension of the culture was possible. After twenty-four hours the tubes were warmed, the media centrifuged to the bottoms of the tubes and cultured. Finally 1 c.c. of fresh medium was inoculated with a loop of the growing cultures. Irradiated mixtures of warmed mineral oil and petrolatum were added and the liquid media were emulsified finely in the oil. The tubes were incubated aerobically or anaerobically according to the requirements of the bacteria. After four days these tubes were warmed and centrifuged. Films and cultures of the sub-

jacent media showed luxuriant growth. The aerobes studied were *Staphylococcus aureus*, a virulent strain of *Streptococcus hemolyticus*, *Neisseria catarrhalis*, *Streptococcus viridans*, a virulent *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Pasteurella lepiseptica*, and *Bacterium coli*. The following Clostridia; chauvaei, histolyticum, novyi, oedematiens, sepique, sporogenes, and welchii, were subjected to the above experiments. None was killed nor was growth apparently inhibited.

## DISCUSSION

The experiments undertaken show conclusively that, as a result of oxidation occurring during exposure of vaseline or liquid petrolatum to ultraviolet light, vaporous peroxides and aldehydes are evolved. Neither the oils nor the vapor from them were sufficiently bactericidal that a germicidal effect on strains of virulent aerobes or stock strains of anaerobes could be demonstrated in vitro. Other oils, according to some authors, when irradiated acquire definite bactericidal properties. They correlate this bactericidal effect with the increase in peroxidic oxygen occurring under irradiation.

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# BACTERICIDAL ACTION OF SHORT AND ULTRASHORT WAVES*

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THE work of foreign authors has shown a specific bactericidal action of ultrashort waves. This means that the electronic action of these ultrahigh frequencies has a lethal action on bacteria because of the peculiarities of the frequency itself and not because of any other effects as induced heat. Furthermore, different bacteria respond differently to different wave lengths; a four-meter wave, for example, may not kill a bacteria, the destruction of which may, however, be accomplished by a 15-meter wave. In short, the object of this paper is to determine the specific action of ultrashort waves on bacteria.

*Experiment I.*—Four bouillon culture tubes of *Staph. aureus* were used. Each tube contained 5 c.c. of culture. The tubes were then placed in a glass beaker filled with ordinary tap water. Since the dialectric constant and conductivity of the water is less than that of the bouillon, the tap water would be exerting no shielding action. A clear bouillon tube was also added, and its temperature was taken to indicate the temperature rise of the bouillon cultures. The condenser plates in all these experiments were put one inch from sides of the glass beaker. Ten meter wave------200 watts------Temperature of bouillon cultures and tap water at start was 33° C. As the temperature of the water began to rise part was drained off and cold water to the same amount added. In this way the temperature of both the water and bouillon did not vary more than 2° C. during the course of the exposure. This means of controlling the temperature rise was used in all the experiments.

| | |
|---|---|
| Tube 1 | Exposed fifteen minutes |
| Tube 2 | Exposed thirty minutes |
| Tube 3 | Exposed forty-five minutes |
| Tube 4 | Exposed sixty minutes |

Subcultures were then made and active growth noted. On the following day Tube 4 was exposed sixty minutes, subcultures were made and active growth was noted. On the following day, Tube 4 was exposed sixty minutes and active growth was noted. Tube 4 of *Staph. aureus* had therefore three hours of exposure on three successive days and no effects were noted.

*Experiment II.*—The above experiment was done with streptococcus in chains, ten meters 200 watts. Tube 4 of the streptococcus, after a total of three hours of exposure on three successive days, showed active growth.

*Experiment III.*—The above was done with typhoid bacilli, 10 meters, 200 watts. Tube 4 after three hours of exposure on three successive days showed active growth.

*Experiment IV.*—The above was done with pneumococcus, 10 meters, 200 watts. Tube 4 after three hours of exposure on three successive days showed active growth.

*Experiment V.*—The above four cultures were exposed on three successive days for a total of three hours to 15 meters, 200 watts. Subcultures showed active growth in all four.

*Experiment VI.*—The above four cultures were exposed for three hours on three successive days to 6 meters, 185 watts. Subcultures again showed active growth.

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Acknowledgment is made to the Elmer and Amend Company for their kind cooperation in the above experiments.

*Experiment VII.*—The above four cultures were exposed to 6 meters, 300 watts for a total of two hours on two successive days. Subcultures again showed active growth.

From the above experiments we can say that short and ultrashort waves in the ranges indicated have no specific action on bacteria in bouillon cultures.

*Experiment VIII.*—Agar plates having cultures of *Staph. aureus* and steptococcus in chains were now put in the condenser field. It was important here to prevent heating of the plates above the lethal temperature of the bacteria. A precision thermometer was placed between two agar plates in the field and in this way the temperature of the plates was found. This of course did not give the absolute temperature of the agar cultures but sufficed to help in keeping the temperature below the lethal point of the bacteria. When the thermometer read 37° C., the agar plates were removed from the field, cooled and then exposed again. In this way the plates were exposed for a total of two hours. They were exposed to 10 meters at 200 watts, and 15 meters at 200 watts. Subcultures were then made and active growth noted.

Our experiments failed to show a bactericidal action of short and ultrashort waves, limited to the respective wave lengths and power used. Of course the body is not a test tube, and in the body certain secondary effects from the use of short waves may have a decided influence upon bacterial life.

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## THE DOCTOR AS AUTHOR*

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THE profession of medicine peculiarly fits the physician for the profession of letters. His practice constantly affords him the opportunity to study and observe a wide miscellany of human characters and the conditions which formed and sustained each individual type. The physician and the novelist both devote their energies in an endeavor to fathom the mysteries of life and to understand them. It was Collins who said: "The novelist is concerned with the psychological details of personality, emotional states and behaviour; the springs of human action must be his incessant study, for his business is to explore human nature and to chart his discoveries, a large part of the physician's life also."

A great many physicians have gained fame in literature, perhaps more than in any other calling, although it is interesting to recall that Richard Jordan Gatling, inventor of the Gatling gun, David Livingstone, who penetrated the depths of Africa, Leander Starr Jameson of the infamous or famous Jameson Raid into Transvaal, Leonard Wood, soldier and ruler of provinces, and Theodore Newton Vail of telephone fame, were all physicians.

Rabelais, immortal author of the classics *Pantagruel* and *Gargantua*, was born in 1490, and studied medicine at the University of Montpellier. In 1530, he received his Doctor of Medicine. For many years he was a busy practitioner

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in Lyons, but he found time to add to his vast fund of erudition and to write the adventures of Pantagruel, which forever will live as great literature.

Rabelais' stories are notable because they are a mixture of insane laughter and mock gravity, because they point out the childishness and grandeur of the commonplace. Rabelais was the first great laugher in literature. He saw life in all its sordid details, and he could not be serious about it.

Among the most notable litterateurs in the dawn of German literature was Friedrich von Schiller, who was born in 1759, and whose plays, *The Robbers, William Tell, Marie Stuart, Maid of Athens,* are among the greatest in the German language. He was graduated in medicine, and for several years was a military surgeon. His success in literature, however, caused him to abandon his medical practice in order to devote more time to his novels and dramas.

One of the greatest poets in any language was John Keats. Few people know that Keats was a qualified physician. He was born in 1795 and during his youth became apprenticed to Thomas Hammond, a prominent surgeon in Edmonton. For some reason or other, he could not get along with Hammond and broke off his apprenticeship. He, however, continued with his medical studies and became a dresser at Guy's Hospital. While at the hospital Keats made the acquaintance of Leigh Hunt, Benjamin Haydon, and Shelley, whose company and ideals he found more congenial than those of his medical associates. It was in 1816 that Keats definitely abandoned medicine, for which he had little taste; two years later he published *Endymion,* which won him imperishable fame.

Dr. John Brown, born in 1810, was not only a Scotch doctor with a large practice, but a talented man in many directions. He was graduated in medicine from Edinburgh and enjoyed practice among his people. He belongs to that group of authors who become known as "one-book" authors, that is, their fame rests wholly on but one book. Dr. Brown is immortalized in literature as the author of the greatest of all dog stories: *Rab and His Friends.*

Oliver Goldsmith, one of the glories of English literature, author of the classics *The Deserted Village* and *The Vicar of Wakefield,* was duly graduated as a doctor and even wrote two medical books. He was of unstable disposition and constantly getting in and out of difficult situations. He tried not only medicine as a means of gaining a livelihood, but also several other professions, and in none was he successful. At length he determined to try his fortune in America. While at a convivial gathering he enjoyed himself more than was wise for him, and his ship sailed without him.

He then decided to study law for which purpose he borrowed fifty pounds. He never realized this ambition because he promptly lost his fifty pounds at a gambling table. At the age of twenty-four, somewhat sobered, he went to Edinburgh to study medicine. For some strange reason or other he was able to get through his courses. But it was not long before his old restlessness returned. With a flute for his companion, he vagabonded over Flanders, France, and Switzerland. He returned at length to England to settle down, and for awhile he eked out a meager existence by pounding drugs and running errands for apothecaries. A little later he was successful in securing a medical appointment with the East India Company, but it was revoked. He also took examinations for a hospital

post, but failed to pass them. Finally he turned to literature, but his monetary returns from this new venture were hardly sufficient to keep him going. He was about to be evicted when he sent for Dr. Johnson. The latter responded, looked over his manuscripts, and was particularly impressed with *The Vicar of Wakefield* whose merit he realized. He sold this manuscript for six pounds to a publisher and gave the money to Goldsmith. He continued to write and later turned out a play, *She Stoops to Conquer*, which did much in establishing Goldsmith's reputation as an author.

Among early American authors, Oliver Wendell Holmes was almost as famous a physician as he was a man of letters. He coined the word "anesthesia" shortly after Dr. Morton's demonstration at the Massachusetts General Hospital. His contribution to the study of puerperal fever and its prevention was as notable as that of Semmelweiss. For forty years he taught anatomy at the Harvard Medical School and during that time he turned out his *Autocrat at the Breakfast Table* as well as other notable novels, poems, and essays.

Charles Joseph Lever, author of *Charles O'Malley*, *Tom Burke*, and others, was born in 1806. His was a wild and stormy life. He ran away to Canada as an unqualified surgeon on an emigrant ship, and for awhile lived with a tribe of Indians. He finally returned to England and was appointed dispensary doctor at Port Steward.

Tobias Smollet, one of the most popular novelists of his day, was a physician. He gained immortality as one of the earliest of great English novelists. Samuel Smiles was another of the "one-book" authors. He was not very successful in practice and finally turned to literature. He wrote a number of books, but his *Self-Help* is the book on which his fame rests. It is one of the everlasting best sellers.

The minor poets and writers had a great many physicians among them. Among the two most notable were Thomas Lovell Beddoes and George Crabbe. Beddoes was born in 1803 and studied medicine at Oxford. He practiced his profession on the Continent and later took an active part in politics. Toward the latter part of his life his interest in literature grew and he wrote *Death Jest Boom* which was published one year after he died.

George Crabbe was born in 1754 and for awhile practiced medicine in his native city of Addeburgh in Suffolk. He was not very successful and went to London to devote himself entirely to literature. He soon established a reputation as being the greatest of English realists.

Sir Arthur Conan Doyle, almost as soon as he began to practice medicine in Southsea, began to write the tales which made Sherlock Holmes a household word throughout the world. He was unsuccessful in obtaining a hospital appointment. But his interest in medicine persisted. He specialized in diseases of the eye, but still success was slow in coming. Meanwhile he continued to write his Sherlock Holmes stories, and their success made him turn seriously to literature, abandoning medicine altogether. His venture in serious writing, while not popularly appreciated, helped to gain him great honors. He was particularly interested in history, and his historical novels, which he considered

much superior to his Sherlock Holmes stories, have never been as popular as they deserve to be. Even more popular were his scientific stories featuring Professor Challenger. Professor Challenger, like Sherlock Holmes, had his counterpart in real life. He was Professor William Rutherford, the physiologist. For his *History of the Boer War* he was knighted. When he lost a son in the World War his interest turned to spiritualism which resulted in a steady stream of spiritualistic books from his pen.

Silas Weir Mitchell was another of the popular American novelists who was also a great doctor. He wrote voluminously on both medical and popular topics. In medicine he became known as the originator of the "rest-cure" and in popular literature he gained fame as the author of a very successful novel, *Hugh Wynne.*

Russia produced one of the most glorious of the doctor-authors, one of the greatest of short story writers, Anton Chekhov. Chekhov practiced medicine during the early part of his adult life and was rather successful, but tuberculosis caused him to curtail his medical activities. He was already making a name for himself as a writer and dramatist. In his short existence he produced some of the greatest stories and dramas which even today are produced throughout the world with success.

John McCree is a poet whose fame will rest on one great poem he wrote, perhaps the greatest produced by the World War. He was born at Guelph, Ontario, Canada, and studied medicine at Toronto. In 1899, he went to McGill University to assume his duties as a pathologist, a little later becoming a lecturer in clinical medicine. In October, 1914, he went overseas as a medical officer with the first Canadian contingent. Four years later he was appointed a consulting physician to the British armies in the field, but before he could enter on his new duties he contracted double pneumonia, from which he died. His great poem, *In Flanders Fields,* was published in *Punch* on December 8, 1915.

The late Poet Laureate of England, Dr. Robert Bridges, was a physician who wrote some excellent medical papers before he turned to writing poetry. He left behind some of the greatest of recent English poems. Dr. Henry Drummond, popularly designated, though unofficially, the Canadian Poet Laureate, practiced medicine in Montreal and elsewhere in Canada for more than twenty years before he finally gave up his practice to describe the life and character of French habitant life in his poems, which have attained a very wide vogue.

The doctor has contributed and is contributing to current literature with greater glory than ever before. The contemporary doctor-author is a very important part of living literature. Perhaps the greatest of living physician-writers is Somerset Maugham, whose plays and novels are now among the classics. Maugham was born in Paris in 1874, the son of the attorney to the British Embassy. He received his education at King's College, Cambridge, and the University of Heidelberg. It was his intention to become a painter, but when his family returned to England he seemed to have lost this ambition and instead determined to study medicine. He received his medical degree but never practiced. His first novel, *Liza of Lambeth,* was based on his experiences in the hospital in which he secured his medical education, but the novel didn't make

much of an impression. This should perhaps have discouraged Maugham from using his own experiences as the basis of a novel, but it didn't. He felt that he had an interesting story to tell, the story of a medical student with a physical handicap, and what life meant to him. He told his story in *Of Human Bondage*, and this proved to be the greatest novel he ever wrote, and a work of fiction which became a classic. He later turned to play writing, in which field he became as popular as in the writing of novels.

Francis Brett Young is another of the older English physician-authors whose greatest novel *My Brother Jonathan* like *Of Human Bondage* deals with medical life. *Portrait of Clare* and *Mr. and Mrs. Pennington* are two other novels of Dr. Young which have proved very popular. Another author belonging to the same group is Warwick Deeping whose greatest novel, *Sorrell and Son*, also deals with medical life. Dr. Deeping is the son and grandson of physicians. He received his medical training at Cambridge but was more interested in literature than in medicine. During the War he served in Gallipoli and France in the Royal Army Medical Corps. After the War Dr. Deeping devoted himself exclusively to fiction writing, producing *The Ten Commandments, Stories of Love and Courage, Ropers Row* and others.

Within very recent years another doctor has joined the group of English doctor-novelists. He is Archibald Joseph Cronin, whose first novel, *Hatter's Castle*, became a world best seller. A second novel, *Three Loves*, although not as popular, also attained world-wide fame. Dr. Cronin was born in Cardross, Scotland, and received his Doctor's degree at Glasgow. He served during the War in the medical corps and later did medical research in Wales for the Department of Mines. After a very busy practice in London his health became undermined and he turned to writing as a recreation. He was so successful as a novelist that he determined to devote all his time to writing.

One woman physician has attained a reputation as a writer with a novel *Doctor Serocold*. She is Dr. Helen Johnson who has written under the name of Helen Asthon. She received her medical training at London University, but after her marriage she retired from practice to devote herself to writing. Besides *Doctor Serocold* she has written *Far Enough* and *A Background for Caroline*.

The best writers of detective stories have been physicians. When Sir Arthur Conan Doyle died his mantle descended upon the shoulders of Dr. Richard Austin Freeman, creator of Dr. Thorndyke, whose adventures are known to millions. Many believe that Dr. Freeman is the greatest of modern detective story writers. At eighteen he began the study of medicine at Middlesex College, and only six years later was made a Fellow of the Royal College of Surgeons. After an attack of tropical fever contracted while in Africa, he returned to England to resume his practice, but his health, always very poor, did not permit it. He turned to writing and particularly to creating detective mysteries which have carried his name throughout the world.

Among American authors there are several who deserve mention. Dr. John Rathbone Oliver, both priest and doctor, has written some of the best

novels of today. His *Victim and Victor* and *Fear* were best sellers. Dr. Oliver leads a very satisfying life. He is a psychiatrist, a priest, a professor of medical history at Johns Hopkins University, and a novelist of great power.

A unique American author-doctor, Charles Alexander Eastman, a full-blooded Indian, has written some of the best stories of American Indian Life. His books, *Smoky Days, Wigwam Evenings, The Red Hunter* and *The Animal People*, have enjoyed a world-wide popularity.

Among other American physicians who have attained fame as writers are Charles Conrad Abbott, J. A. Markey, Alden Arthur Knipe, James Bayard Clark, C. E. Blanchard, Edward C. L. Adams, and William Carlo Williams. Edward C. L. Adams has written some of the best stories of American negro life. Among his best-known stories are *Congaree Sketches, Nigger to Nigger,* and *Potee's Gal.* William Carlo Williams is known for his two novels, *In the American Grain* and *Voyage to Pagany.*

The glorious tradition of the physician-author is still being carried on by an ever increasing number of physicians throughout the world. In Germany it is Alfred Doblin, author of the world success, *Alexander Platz;* in the United States, Robert McNair Wilson, William H. Holcombe, Arthur Gash and others; in the Philippine Islands it is Jose Rizal, whose *The Eagle's Flight* has been compared in power to *Uncle Tom's Cabin.* The doctor has been among the most significant makers of literature from the earliest times. He still is.

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# BLOOD SEDIMENTATION RATE IN DIABETES MELLITUS*

## An Analytical Study of 510 Tests Performed on 366 Patients

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THE blood sedimentation test has been used in various conditions, particularly in tuberculosis and pelvic inflammatory disease. Its value as a guide in determining the degree of activity of an inflammatory process has been more or less accepted.

The question as to whether this test could be applied in studying diabetes was considered, first, because of the prevalence of low-grade infections in this disease; second, because we wanted to investigate whether diabetes per se had any influence upon blood sedimentation.

Referring to the first problem, we thought it might be of interest to learn whether the commonly accepted focal infections have any systemic influence upon the diabetic individual, employing the blood sedimentation test as a guide.

The second problem was to learn, if possible, what influence diabetes has

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upon the sedimentation rate. Having in mind the tendency of diabetic patients to develop infections because of their lowered resistance, we were curious to know whether or not this tendency could be recognized by studying the index of sedimentation upon a group of diabetics.

*Method of Study and Technic.*—Five hundred and ten tests were made upon 366 patients. Office and out-patient cases embraced this series. They were not selected. A record was made of the duration of the disease, the age of the patient, and the presence of focal infections: such as the condition of the teeth, the tonsils, evidence of sinus infection, gallbladder disease; notation was also made of the presence of other infections such as arthritis, pyelitis, pulmonary diseases, cellulitis and any other conditions which may have some

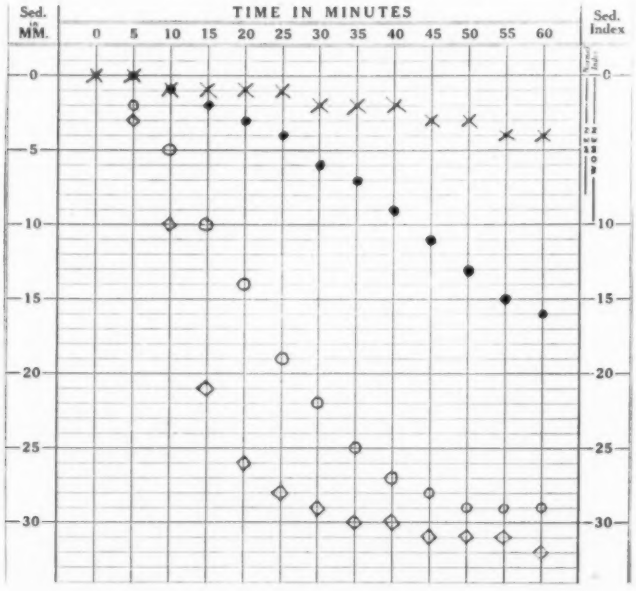


Fig. 1.

influence upon the blood sedimentation. Blood sugar estimations were made at the same time that the blood was obtained for the sedimentation test. Data obtained from this series of tests and observations were recorded and arranged in tabular form. These tables will be discussed later.

The *technic* employed in this study was the one advocated by Cutler.[1, 2] Time and space do not permit a discussion of the various tests employed for blood sedimentation. Blood was obtained by venapuncture. When working with the 5 c.c. tubes, 4.5 c.c. of blood were added to 0.5 c.c. of 3 per cent sodium citrate or if the 1 c.c. tubes are in use, add 0.9 c.c. blood to 0.1 c.c. of the citrate solution. Either test may be employed. The tubes containing the mixture are shaken gently, and then permitted to stand for sixty minutes. Readings are taken every five minutes and the level of the cells is recorded on graphs, graded in millimeters. At the end of the sixty-minute period, the graph may be interpreted.

The resulting curves or lines are classified according to the rate and degree of sedimentation. These may be grouped in the following types:

1. The *horizontal line* drops down slightly but remains within the normal range of 0 to 8 mm. in males, 0 to 10 mm. in females. This is the graph one sees in normals or where the pathologic process, if any exists, is inactive.

2. *Diagonal line:* in which the sedimentation seems to occur at regular intervals, forming a straight line from 0 to the final reading, which is usually between 10 and 20, but may extend lower.

3. *Diagonal curve:* drops faster than the diagonal line, then settles down to a slower period of what is described as "packing." This graph is usually met with when the sedimentation index ranges from 20 to 30 mm.

4. *Vertical curve:* where the red cells settle more quickly, the line dropping acutely, usually reaching a drop of 20 to 25 mm. within the first half hour and then the graph develops a curve of "packing." This type of graph is seen in the acute and more active infections.

For illustration, the above mentioned types are depicted on the sedimentation chart in Fig. 1.

*Diabetes and Blood Sedimentation.*—Of the 510 tests, 164 were of the horizontal line group, 262 were diagonal lines, 76 were diagonal curves, and 8 were vertical curves. The combined abnormal curves were 346 or 67.8 per cent. The horizontal lines were about one-third or 32.2 per cent.

A more detailed study was made of the records. They were arranged according to the type of curve. These different groups were analyzed and figures obtained according to the duration of the diabetes, to the blood sugar, and the age in decades. Results may be seen in Tables I to III.

TABLE I

ANALYSIS OF THE BLOOD SEDIMENTATION RATE ACCORDING TO THE DURATION OF DIABETES

| DURATION | TOTAL CASES | HORIZONTAL | | ABNORMAL | |
|---|---|---|---|---|---|
| | | NO. | PER CENT | NO. | PER CENT |
| Up to 3 yr. | 192 | 66 | 34.4 | 126 | 65.6 |
| 3 to 5 | 104 | 37 | 35.6 | 67 | 64.4 |
| 6 to 10 | 123 | 36 | 29.3 | 87 | 70.7 |
| 11-up | 86 | 25 | 29.1 | 61 | 70.9 |
| Not mentioned | 5 | – | | 5 | |

The percentages in both groups seem to vary only slightly. The difference between the early diabetic patients (34.4 per cent) and those who have had diabetes eleven years or more (29.1 per cent) is only 5.3 per cent. This difference does not seem significant enough to indicate any influence of the duration of the disease upon the sedimentation rate, because one can surely attribute this disparity in figures to age and the possibility of infections developing in older people.

The next consideration of the diabetes-sedimentation problem was the study of blood sugar estimation at the time when the sedimentation tests were performed. The results are arranged in Table II.

If we separate the above records into Group A, embracing the sedimentation rates of those who had a blood sugar of 180 mg. or below, we find that

69 cases or 31.2 per cent showed horizontal lines and 152 records or 68.8 per cent had abnormal ones. Comparing this with the hyperglycemia Group B, we find that 95 showed horizontal lines (32.9 per cent) and 194 (67.1 per cent) had abnormal lines. Another interesting fact: if we single out for illustration, the 15 mm. diagonal line series which showed the largest individual total

TABLE II

SHOWING THE BLOOD SUGAR IN MG. PER 100 C.C. COMPARED WITH THE INDEX OF SEDIMENTATION

| BLOOD SUGAR MG. | HORIZONTAL NORMAL | DIAGONAL LINE | | | DIAGONAL CURVE ABNORMAL | VERTICAL CURVE | TOTAL ABNORMAL |
|---|---|---|---|---|---|---|---|
| | | 15 | 20 | 25-30 | 15-30 | 25-35 | |
| -120 | 20 | 23 | 16 | 4 | 8 | – | 51 |
| 121-150 | 35 | 22 | 16 | 1 | 8 | 2 | 49 |
| 151-180 | 14 | 15 | 12 | 6 | 17 | 2 | 52 |
| Total: below 180 | 69 | 60 | 44 | 11 | 33 | 4 | 152 |
| 181-200 | 25 | 13 | 18 | 4 | 5 | 2 | 42 |
| 201-250 | 34 | 26 | 28 | 5 | 6 | – | 65 |
| 251-up | 36 | 24 | 21 | 8 | 32 | 2 | 87 |
| Total: above 180 | 95 | 63 | 67 | 17 | 43 | 4 | 194 |

of 123 records, in 60 of these the blood sugar was 180 mg. or below, while 63 belonged to the hyperglycemic group. One is impressed with the similarity of the percentages in both groups. The difference of 1.7 per cent is striking and seems to be a convincing argument against the effect of the blood sugar upon the sedimentation rate. This deduction concurs with the conclusions of Wisselinck,[3] Remer,[4] Goedel and Hubert.[5]

*Age.*—An analysis of the sedimentation curves in reference to age was studied and arranged in Table III.

TABLE III

SHOWING AN ANALYSIS OF THE BLOOD SEDIMENTATION GROUPS ACCORDING TO AGE IN DECADES

| AGE | HORIZONTAL | | TOTAL | DIAGONAL LINE | | | | DIAGONAL CURVE | | | | VERTICAL CURVE | | | TOTAL ABNORMAL | COMB. TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | | 15 | 20 | 25 | 30 | 15 | 20 | 25 | 30 | 25 | 30 | 35 | | |
| to 20 | 7 | 3 | 10 | 2 | 2 | -- | -- | -- | -- | -- | -- | -- | -- | -- | 4 | 14 |
| 21-30 | 7 | 9 | 16 | 6 | 3 | -- | -- | -- | -- | -- | -- | -- | -- | -- | 9 | 25 |
| 31-40 | 5 | 8 | 13 | 11 | 9 | 2 | -- | 1 | -- | -- | -- | -- | -- | -- | 23 | 36 |
| 41-50 | 15 | 39 | 54 | 32 | 33 | 5 | -- | -- | 6 | 10 | 1 | -- | 1 | -- | 88 | 142 |
| 51-60 | 5 | 31 | 36 | 44 | 30 | 13 | -- | -- | 8 | 16 | 7 | 1 | -- | 3 | 122 | 158 |
| 61-up | 10 | 25 | 35 | 28 | 34 | 6 | 2 | 1 | 4 | 15 | 7 | 1 | 2 | -- | 100 | 135 |
| Total | 49 | 115 | 164 | 123 | 111 | 26 | 2 | 2 | 18 | 41 | 15 | 2 | 3 | 3 | 346 | 510 |
| | 164 | | | 262 | | | | 76 | | | | 8 | | | | |

We noticed that the younger patients coming under our observation showed either horizontal lines or the slightly abnormal curves. Since diabetes is a disease which occurs more commonly in the fifth, sixth, and seventh decades, we naturally would expect to find the greater number of records in the older diabetic patients. However, it was interesting to observe that the abnormal rates were found in greater numbers proportionately with the in-

crease in the age groups. We are not prepared to offer the exact cause of this observation, but we presume that the reasons for the abnormal sedimentation rates in the aged are most likely due to cell destruction resulting from wear and tear of the tissues and the fact that the aged are more liable to develop infections which may exert some influence upon the rate of sedimentation.

*Infections.*—An analytical study was made of the various records for the presence of infections. The results are arranged in Table IV.

TABLE IV

SHOWING THE PREVALENCE OF INFECTIONS IN THE VARIOUS SEDIMENTATION GROUPS

| | HORIZONTAL | | DIAGONAL LINE | | | | DIAGONAL CURVE | | | | VERTICAL CURVE | | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | 30 | 15 | 20 | 25 | 30 | 25 | 30 | 35 | |
| Teeth: | | | | | | | | | | | | | | |
| Good | 16 | 14 | 16 | 3 | | | | | 1 | | | | | 50 |
| Bad, pyorrhea | 2 | 12 | 12 | 15 | 7 | | | 1 | 9 | 2 | 1 | 1 | 2 | 64 |
| Alveolar abscess | | | 1 | 1 | | | | | | | | | | 2 |
| Missing | 13 | 53 | 56 | 61 | 16 | | | 10 | 27 | 12 | | 12 | | 260 |
| Tonsils: | | | | | | | | | | | | | | |
| Enlarged | 7 | 28 | 15 | 21 | 6 | | | 3 | 6 | 3 | | 1 | | 90 |
| Diseased | 22 | 60 | 52 | 61 | 15 | | | 7 | 17 | 3 | 1 | 2 | 1 | 241 |
| Pharyngitis | 20 | 32 | 33 | 23 | 4 | | | 1 | 15 | 1 | | | | 129 |
| Sinusitis | 5 | 8 | 13 | 18 | 1 | | | 2 | | 1 | | 1 | | 49 |
| Colds (upper respiratory) | | | 6 | 5 | 1 | | | 1 | 1 | 1 | | 1 | | 16 |
| Tuberculosis: | | | | | | | | | | | | | | |
| Clinical | 1 | | 4 | 10 | 1 | | | | 2 | | | | | 18 |
| Proved | | | | | | | | | 1 | 1 | | | | 2 |
| G. B.: | | | | | | | | | | | | | | |
| Suggestive | | 6 | 12 | 16 | 1 | | | 1 | 4 | 1 | | | | 41 |
| Definite | 2 | 5 | 2 | 6 | 2 | | | 5 | 2 | | | | | 24 |
| Renal: pyelitis, cystitis | 1 | 8 | 6 | 1 | | | | | 1 | 2 | | 2 | | 21 |
| Cellulitis and boils | | 5 | 3 | 4 | | | | | 3 | 2 | | | | 17 |
| Arthritis and osteoarthritis | 1 | 9 | 10 | 6 | 3 | | | | 4 | 2 | | 1 | | 36 |

One meets with obstacles when attempting to interpret the results of the sedimentation tests in the presence of minor infections. Sufficient work has not been done along these lines to furnish us with guides. Then again, the presence of combinations of focal infections will prevent us from definitely isolating the chief offender.

It is not our intention to become involved in a discussion of infections in diabetes. We were chiefly interested to learn whether the sedimentation will drop to abnormal ranges in patients with bad teeth, diseased tonsils, sinus conditions, etc.

In this table one may notice that a vast majority of the patients with bad *teeth* are in the abnormal groups. Most of the *pyorrhea* cases behaved similarly. However, a fairly high percentage of the patients with good teeth showed abnormal lines. We must bear in mind what was mentioned above, namely, that there may be other forces besides the teeth which may affect the sedimentation rate in these patients.

In the *tonsil* series, it was noticed that about one-third of the records of patients with diseased tonsils showed horizontal lines. In the remaining cases we found abnormal drops in sedimentation. It may be possible that the absorption from the diseased tonsils had some effect upon blood sedimentation.

The question of the effect of *sinus* infection holds a similar position as the tonsils. Of the 49 records of patients with a history of sinus disease, 13 showed horizontal lines; the balance of 36 had abnormal lines (73.4 per cent). While in this group we may consider those patients with upper respiratory *colds:* 16, the entire number, showed abnormal lines, 11 of them belonging to the milder diagonal group.

In the pulmonary diseases, the *tuberculosis* series, 19 of the 20 had abnormal lines. This phase of the subject need not be discussed because many contributions have been published on the clinical value of blood sedimentation in tuberculosis.

*Gallbladder* disease showed a vast majority in the abnormal ranges.

In *pyelitis* and *cystitis*, abnormal lines were present in slightly over 50 per cent.

In the series of *arthritis* and *osteoarthritis*, 26 of 36 tests showed abnormal lines. However, one must keep in mind the presence of focal infections in these patients and their possible effect upon the sedimentation rate.

## SUMMARY AND CONCLUSIONS

A study of 510 sedimentation tests was performed upon a group of 366 patients; 346, or 67.8 per cent, showed abnormal readings.

The explanation for this high percentage must be either the diabetes or the presence of infections, or both.

A study of the duration of the diabetes showed that the maximum difference of the percentages in the various diabetic groups was only 5.3 per cent. These figures evidently do not permit us to attach much significance to the effect of the duration of the diabetes upon the sedimentation rate.

The possible influence of the blood sugar was also studied. There was a striking similarity of the percentages in the hyperglycemic group and those whose blood sugar was 180 mg. or below: in the former there were 194 cases (67.1 per cent) which showed abnormal lines, and in the latter (nonhyperglycemic group), there were 152 records (68.8 per cent) with abnormal lines. From our studies we concluded that the blood sugar per se had no influence upon the sedimentation rate.

Since the duration of the diabetes and the blood sugar cannot explain the high incidence of the abnormal sedimentation rates, the inference is that the most likely explanation is infection. Analysis of the patients observed in this study showed a high incidence of the minor infections, particularly in the teeth, tonsils, and upper respiratory tract. However, the urinary tract and gallbladder must also be kept in mind. The effect of tuberculosis upon sedimentation does not require any comment here.

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# SUBCLINICAL SCURVY IN CHILDREN*

## The Application of the Capillary Resistance Test

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With the Technical Assistance of Richard F. Cousins

AT THE request of the late Dr. Alfred F. Hess, of New York City, a study was undertaken in the New Jersey State Home for Boys to determine the specificity of the capillary resistance test for scurvy. He considered the diet of our boys adequate in all respects, especially the intake of antiscorbutic foods.

Each boy receives about one quart of milk daily in some form or other. Vegetables are supplied daily from our own farm and truck garden. During the winter months the surplus of vegetables which have either been stored or canned is served the boys. In addition, butter, cereals, and meat are part of the daily diet. Tomatoes are in abundance while citrous fruits are less evident.

The capillary resistance test was first used by Rumpel[1] in 1909 for diagnosing scarlet fever without a rash by means of a Bier compression band placed about the upper arm, thereby producing hemorrhages in the skin. Leede[2] in 1911 further elaborated this test using the Riva-Rocco apparatus. Hess and Fish[3] in 1914 further improved the test and were the first to use a blood pressure tourniquet. Göthlin[4] after several years of experimentation modified and improved the method; we followed his technic in detail without attempting any modification on our part. Dalldorf[5] improved a method of applying negative pressure to the surfaces of the skin through a suction cup connected with a mercury manometer and vacuum pump.

The purpose of the capillary resistance test is to determine the ability of the small blood vessels to withstand increased intravascular pressure which is brought about by means of a tourniquet applied to the arm. In 1920, Hess[6] in his classic treatise on scurvy stated that the test is not specific for this disorder but that it is positive in the majority of cases of scurvy. It has been generally accepted that capillary fragility, leading to the appearance of petechiae, is one of the earliest detectable manifestations of the effects of a scurvy-producing diet in

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animals. Hess[7] in 1921 suggested that a "failure of the integrity of the epithelium of the blood vessels" occurred and that "this was due to a lesion of the endothelial cells or their cement substance." Wolbach and Howe[8] believed that the defect in the capillaries results from a failure of the endothelial cells to form cement substance and they concluded that the essential pathologic alteration in scurvy is "an inability of the supporting tissue to produce and maintain intercellular substances."

Hess[9] further questions the specificity of the test by stating, "in my experience there are decided individual variations in regard to the reaction to this test, so that although it is true that petechial spots are far more numerous in individuals suffering from latent or active scurvy, the reaction cannot be used as evidence of a deficiency in vitamin C intake. Occasionally edema rather than hemorrhage has been found in the scurvy of adults and of infants. The cause of these extravasations, which have been noted most often at the ankles, is not known. It would seem most likely that it is associated with a retention of sodium chloride." Clinical studies of this aspect were published by Meyer[10] in 1923, by György[11] in 1927, by Öhnell[12] in 1928, and by Göthlin[13] in 1931. Öhnell first suggested the use of the test in the recognition of mild forms of vitamin C deficiency.

The method used by us was identical with that developed by Göthlin[4] and the reader is referred to his article for details and procedure. An excellent photograph of the apparatus attached to the patient and ready for use is also found in the above article. Our boys were all tested in the afternoon and in the horizontal position. The arms were in the same plane as the heart to minimize differences of pressure. The room temperature was comfortable and more or less controlled to prevent chilling. The light under which the readings were made was standardized by using an electric lamp with the same size bulb and reflector for all the tests.

A colored ring, of 60 mm. diameter, was imprinted with a rubber stamp on both arms with its center coinciding with the center of the hollow of the elbow. The area of the skin thus outlined was thoroughly inspected through a magnification lens (5D) before pressure was exerted. If any formations which might have later been mistaken for fresh petechiae were visible they were marked for exclusion. The rubber tourniquet was then wound about the arm and fixed, the lower edge of the tourniquet being at least 2.5 cm. above the nearest part of the colored ring. This is important as occasionally petechiae appear in greater number just below the lower edge of the tourniquet. The pressure in the tourniquet is quickly raised to 50 mm. of mercury and kept there by means of a screw compressor. In our work three boys were done at the same time with one mercury manometer. A blood pressure bulb was used to pump the air in and the screw compressor was used to make the finer adjustments. During the fifteen minutes that the pressure was maintained, we found it necessary to make slight adjustments not more than twice during the period. The petechiae were counted within fifteen minutes after the tourniquet was removed and the average of both arms recorded. An average of more than 8 petechiae was con-

sidered "positive" while those between 5 and 8 were called "transitional" and those below 5 were considered "negative."

Göthlin has gone into this subject rather extensively and is convinced that the capillary resistance test is specific for scurvy. He[13] placed two healthy adult demented patients on a scorbutic diet for a few weeks and found that the original normal strength of their skin capillaries fell to an abnormally low level but that this change in the capillary strength was reversible, the strength returning gradually to normal when a sufficient amount of orange juice was given for several weeks. Göthlin mentions that positive results may be obtained in acute infectious diseases and in cases of albuminuria. This we have confirmed and we have also obtained positive results in one boy who has chronic asthma (sensitive to ragweed pollen) and in one boy who had recently recovered from a dermatitis venenata (poison ivy). One boy with ichthyosis of the skin gave a negative result but was not included in our data because we were not sure of our readings.

Our tests were all done between January and May, 1934, the time of the year when the stored amount of antiscorbutic vitamin is probably at its minimum. The boys were free of any acute infections and all of our positive cases were examined in more detail to eliminate any abnormality which might give false positives. Only one boy was found to have a mild gingivitis. Falk et al.[14] found that when the capillary test revealed definite subnormality in the capillary strength and the vitamin C standard, this subnormality was in 70 per cent accompanied by gingivitis, and on the other hand that 10 per cent of those children who were examined and found to have a normal capillary strength, and thus a normal vitamin C standard, nevertheless, exhibited mild gingivitic alteration.

There were 969 tests done on 418 boys as illustrated in Table I. Only averages of the number of petechiae found on both arms are reported. Of the 418

TABLE I

969 TESTS WERE DONE ON 418 BOYS. THE GROUP IS DIVIDED INTO THE LIGHT-SKINNED NORDIC TYPE (L) DARK-SKINNED SOUTHERN EUROPEAN (D) AND THE INTERMEDIATE COMPLEXIONS (I) COMMONLY FOUND AMONG THE AMERICANS

| AGE | NUMBER OF CASES | | | NEGATIVES (1-4 PETECHIAE) | | | TRANSITIONALS (5-8 PETECHIAE) | | | POSITIVES (ABOVE 8 PETECHIAE) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | L | I | D | L | I | D | L | I | D | L | I | D |
| 9 | | 1 | | | 1 | | | | | | | |
| 10 | 6 | 1 | 1 | 4 | 1 | 1 | 1 | | | 1 | | |
| 11 | 4 | 6 | 5 | 4 | 5 | 4 | | | | | 1 | 1 |
| 12 | 6 | 20 | 3 | 5 | 16 | 3 | 1 | 2 | | | 2 | |
| 13 | 4 | 24 | 7 | 3 | 20 | 7 | 1 | 2 | | | 2 | |
| 14 | 11 | 39 | 13 | 9 | 34 | 11 | 1 | 4 | 1 | 1 | 1 | 1 |
| 15 | 17 | 64 | 16 | 14 | 59 | 15 | 2 | 5 | | 1 | | 1 |
| 16 | 18 | 79 | 27 | 17 | 74 | 25 | | 3 | 2 | 1 | 2 | |
| 17 | 4 | 21 | 10 | 3 | 16 | 9 | 1 | 3 | | | 2 | 1 |
| 18 | 1 | 5 | 4 | 1 | 5 | 4 | | | | | | |
| Totals | 72 | 260 | 86 | 61 | 231 | 79 | 7 | 19 | 3 | 4 | 10 | 4 |
| Per cent | 17.2 | 62.2 | 20.6 | 84.7 | 88.9 | 91.9 | 9.7 | 7.3 | 3.5 | 5.6 | 3.8 | 4.6 |
| Grand totals | 418 | | | 371 88.7% | | | 29 6.9% | | | 18 4.3% | | |

boys, 4.3 per cent were found to be positive, 6.9 per cent transitional, and 88.7 per cent negative.

Many boys, without any treatment, were retested at intervals of one or more months and in almost every instance again fell into their original groups. Seventy-one boys gave zero results on both arms, 19 were retested and all fell again into the negative category, but only 7 of these 19 again tested zero on both arms. Fifteen boys who were found to have an average of more than 8 petechiae (positives) were retested, without previous treatment, and only one boy was found to have fallen into the negative group, while 9 were even more positive than before.

Dr. David Greene of New York City brought to my attention the discrepancies he found when testing both arms of his patients. Göthlin[13] tested both arms on 28 individuals and found that the largest difference was 3 petechiae in any one of them. In a small percentage (3.3) we found 14 boys were positive (more than 8 petechiae) on one arm and negative (less than 4 petechiae) on the other arm. Six of these boys were retested a month later and approximately the same differences were obtained. In 5 boys we found positive results on one arm and transitional (between 5 and 8 petechiae) findings on the other arm. In our group we found that 34 boys or 8.1 per cent had 8 or more petechiae on one arm but only 18 or 4.3 per cent had an average of 8 or more petechiae for both arms. If only the right arms were tested, 28 boys or 6.7 per cent would have been positive, while if only the left arms were tested, 22 boys or 5.2 per cent would have been positive. Therefore our lowest incidence of positives (4.3 per cent) was obtained when the averages of both arms were used. This is just the reverse of what Greene[15] found in a small group of cases. If a small group is being studied the authors recommend that both arms be tested but in very large groups, especially where the presence of scurvy is anticipated, it is suggested that only one arm be tested, preferably the left (Table II).

TABLE II

COMPARISON OF RESULTS FROM THE USE OF ONE ARM AND OF BOTH ARMS

| | AVERAGE OF BOTH ARMS | LEFT ARM ONLY | RIGHT ARM ONLY |
|---|---|---|---|
| % Positive | 4.3 | 5.2 | 6.7 |

There appears to be no relation between handedness and the development of petechiae. In our group 21 were left handed (5 per cent) and the remainder right handed. Of the left-handed boys 10 had more petechiae on their right arm, 5 had more on their left arm, while the remainder had equal numbers. Of the right-handed group there was a slight but negligible predominance of the number of petechiae found on the right arm.

Dalldorf[5] believes that the character of the skin itself partly determines the results of the tests. The groups studied included dark-skinned southern Europeans, light-skinned Nordic types, and persons with intermediate complexions, commonly found among Americans. Table I gives the results of this differentiation. The light-skinned group gave a slightly higher incidence of positives, and

there is nothing in our data to support Dalldorf's contention that the character of the skin, in relation to the intensity of pigment, predetermined partly the results of the tests.

Hess[9] stated that, "Economic conditions have, however, always had an effect on the incidence of scurvy, and it is quite possible, if not probable, that the economic depression in this country and abroad may entail an increase in latent and manifest scurvy in adults as well as infants." Dalldorf[5] recommends that the dietary of the poorer families be studied. Our boys are all committed by the courts of the State of New Jersey because of juvenile delinquency. These boys, in a large percentage, come from very poor homes where the diet is irregular and uncertain. Many of these families are "on relief" from the federal, state, or municipal governments. Fifty-six boys were tested within a day or two of their admission to the institution. Forty-nine were found to be negative, six transitional, and one positive. In spite of supposed poor dietary and the presence of dental caries (in almost every boy on admission), the positive results are even less than in our population as a whole. Incidentally in a study made over a period of three years, we found that our newly admitted boys were also well nourished. Fifteen of our so-called psychopaths who live in a separate unit were tested and all were negative except one transitional.

In an analysis of Table I it is seen that 4.3 per cent were found to be positive. This is an extremely low figure when we compare the data obtained by others. Göthlin[13] found 18 per cent positives among school children between the ages of eleven and fourteen, while we found 6.3 per cent positives within this age group. He[14] later studied 3 school groups in different localities and found that 21.5, 5.9, and 19.4 per cent were positives. Where the low percentage was obtained, he found that the diet revealed the highest number of guinea pig units of vitamin C. Dalldorf[5] reported from 35 to 66 per cent positives, with the suction method, in children from poor homes. Greene[15] in a group of 65 children between the ages of nine and fourteen years found an incidence of 9.2 per cent positives. Within this age range we found an incidence of 6.6 per cent positives. Our low results are most probably due to the adequate diet furnished the boys.

A small group of boys, 5 transitionals and 10 positives, were retested at least twice and then treated for three weeks with about 6 ounces of concentrated canned tomato juice daily. Of the positives, 2 remained positive, 2 became transitional, while the remainder became negative. Of the transitionals all became negative except one who remained in the same category. The above two positives were then given the juice of one orange daily for three weeks, and one became negative while the other was tested but not read because of an acute dermatitis venenata (poison ivy). Three additional boys, who had received tomato juice with subsequent negative results, were further treated with orange juice with interesting findings; one remained negative while the remaining 2 became positive and more so than before treatment with the tomato juice. The presence of other factors causing this bizarre result was not ascertained. Five boys with positive findings, who had not received tomato juice, were treated with

orange juice for three weeks and all became negative except one who remained positive.

## SUMMARY

There were 969 capillary resistance tests performed on 418 boys to determine the specificity of the test for scurvy and the incidence of positive results before and after treatment with tomato and orange juice. The method employed was the one improved by Göthlin of Upsala, Sweden. Such factors as acute infections, albuminuria, use of both arms, handedness, and color of skin were considered. Boys recently admitted to our institution from poor families were tested to ascertain the adequacy of their diets in relation to antiscorbutic contents.

## CONCLUSIONS

1. The incidence of positive reactions was 4.3 per cent in 418 boys on whom 969 capillary resistance tests were done. This low incidence is probably due to the adequate diet given our boys in the institution.

2. The color of the skin (light Nordic type, dark-skinned southern European type, and the intermediate complexions found among Americans) had practically no relation to the results obtained.

3. There appears to be no relationship between handedness and the development of petechiae on either arm.

4. In testing small groups it is recommended that the averages of both arms be used.

5. Retests, without treatment, proved the test to be consistent in its results.

6. The lowest incidence of positive results was obtained in fifty-six boys who were recently admitted from the "poorer" homes, i.e., from an economic standpoint.

7. A small group of boys, 15 positives and 5 transitionals, were treated with tomato and orange juice and all but 3 cases were benefited.

8. Although we do not consider this test as specific, we nevertheless do think that it is the best available method for the recognition of the subclinical cases of scurvy.

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# EXPERIMENTAL ARTHRITIS IN RABBITS*

## Comparison of the Arthritis-Producing Ability of Inagglutinable Streptococci Which Resist the "Bactericidal" Action of Fresh, Diluted, Defibrinated Guinea Pig Blood and Those Which Are Agglutinable But Sensitive to the "Bactericidal" Agent

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ALTHOUGH considerable work has been done on the etiology of rheumatoid arthritis, it is still an unsettled problem. Certain workers have reported the isolation of "specific" strains of streptococci from the blood of patients with rheumatoid arthritis, but others have been unable to confirm these claims. Our own series of 100 blood cultures failed to reveal bacteria except those of the type reported by Callow.[1] In spite of these conflicting reports, it is generally believed that some type of streptococcus plays an important etiologic rôle. In view of the unsatisfactory results usually obtained, other methods were sought for the isolation and differentiation of streptococci obtained from patients suspected of harboring strains of etiologic importance.

In the present paper, in addition to discussing isolation methods, we shall compare the ability to produce arthritis in rabbits of strains which either (1) were agglutinated by the patient's blood serum or (2) were resistant to the "bactericidal" action of fresh, diluted, defibrinated guinea pig blood or (3) reacted positively or negatively to both tests. It seemed advisable to include a technic for (1) the selective isolation of strains which are more likely to be of etiologic importance and (2) the intensification of differences in the appearances of colonies (because of the possibility that these differences might be associated with important pathologic properties).

Because of the difficulties of preparing stable emulsions, rough strains of streptococci are less suitable for quantitative study. Therefore, they have not been considered in the present report. This becomes important when it is considered that, although the pathologic significance of the difference be-

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tween rough and smooth strains is not clearly understood, it is generally considered that smooth strains are more pathogenic for laboratory animals than rough ones. Cowan,[2, 3, 4] Tunnicliff,[5] and Delves[6] demonstrated that the rough strains which they studied were less "virulent" for mice and rabbits than smooth strains.

The original method used for the selective isolation of smooth strains consisted of plating swabs on blood agar, both aerobically and anaerobically, and isolating and purifying the various types of colonies. Of 1,164 strains of streptococci secured in this manner, 470 (40.4 per cent) were rough and many swabs yielded no smooth strains whatsoever. In certain cases it was necessary to make several consecutive daily examinations before a suitable strain was obtained. Nickel's[7] observation that 59 per cent of the cultures from prostatic secretion did not grow aerobically in primary culture, such as an ordinary streaked blood agar plate or a tube of nutrient broth, prompted a search for better methods of isolation.

It was postulated that, since rough strains tended to form a sediment in broth or other fluid medium, and since streptococci were found to grow better in Rosenow's brain heart infusion, a predominance of smooth strains should be obtainable from the upper layers of brain heart infusion cultures after incubation of the swabs directly in the medium. A group of 284 strains was isolated from blood agar transplants from these upper layers, and only 54 (19 per cent) proved to be rough. Many of the rough strains probably originated from floccules of culture which had been dislodged from the swabs. To eliminate them, the swabs were withdrawn from the tubes after overnight growth and the cultures shaken and allowed to stand in the incubator for an hour or so. This permitted settling of the rough strains. Transplants were made from the supernatant suspensions and the proportion of rough strains was found to be still further reduced. Of 3,500 strains isolated in this manner, only 375 (10.7 per cent) were rough. In a second series, comprising 6,028 strains, 453 (7.4 per cent) were rough. A still more recent series of 2,839 strains contained 131 (4.5 per cent) rough strains. If smooth strains of streptococci were not recovered from the culture, further swabs were examined.

Several points in technic require amplification. Under ordinary working conditions in the average clinical laboratory, a large proportion of streptococcal cultures are impure. In an effort to minimize this defect, the various steps usually employed were investigated, and it was found that several of them invited contamination. Much of it, attributed to air-borne bacteria, was eliminated by the installation of an air filter. However, contaminations continued to occur. It was recalled that, in order to inoculate a Petri dish of culture medium with a platinum loop, it was necessary to make a large number of strokes to cover the plate. This made it difficult to avoid contamination. To overcome this a "spreader" was used. It consisted of an 8-inch glass rod with the rounded end bent at a right angle 4 cm. from the end. These spreaders were sterilized in long manila envelopes with open ends, sealed by gummed flaps. When ready for use the handle end of the envelope was torn off and the spreader lifted out, taking care to avoid contamination of the

bent end from contact with the exposed edges of the envelopes. Three or four strokes of the spreader were sufficient to cover the whole plate.

The second source of contamination was found in the method used to inoculate the liquid medium. A platinum needle was not found satisfactory for harvesting the growths on blood agar because it left the culture piled into a series of ridges. On account of the large number of strokes necessary, it was difficult to avoid contamination. A scraper was devised for obtaining the maximum amount of inoculum from the plates with a minimum number of strokes. This scraper consisted of an aluminum rod used for a handle, and an aluminum blade which was just wide enough to pass through the neck of the bottle containing liquid medium.

With these two instruments and by ventilating the laboratory with filtered air, contamination was reduced to less than 2 per cent of the strains. Growths in the liquid medium were always tested for purity and bile solubility.

When the blood agar plates were incubated in an anaerobic jar, it was noted that the anaerobic environment had intensified the green color produced by certain strains and had favored the development of green pigment by certain strains which did not reveal any when grown aerobically. Furthermore, certain strains which produced hemolytic zones aerobically failed to do so anaerobically but developed green zones instead. It was concluded that the anaerobic method of cultivation permitted a better differentiation of streptococcal colonies than was possible by the aerobic method. It was further noted that, when an aerobic culture appeared to be a pure growth, anaerobic cultivation often revealed several different types of colonies. On certain blood agar plates, no colonies were detected by casual examination, but growths were discovered on careful study of the surface of the medium.

The technic, as finally adopted, was as follows: The swabs were placed in tall tubes of brain heart infusion and incubated overnight. The following morning the swabs were withdrawn and the tubes gently rotated. They were then allowed to stand in the incubator for one hour. A loopful of the supernatant growth was transplanted to blood agar, spread by means of the glass spreader, and incubated overnight in an anaerobic jar. The following morning the growths were critically examined by transmitted and reflected light. Representative types of colonies were selected, transplanted to other blood agar plates, and incubated overnight in an anaerobic jar. Impure cultures were further purified.* The pure substrains were harvested by means of the metal scrapers and transferred to brain heart infusion. Growths usually occurred in less than twenty-four hours but, if the growth in any bottle was poor, it was returned to the incubator.

Cecil et al.[8] reported that the serum of patients with rheumatoid arthritis agglutinated certain "typical" strains of streptococci which they had obtained by blood culture. Believing that the agglutination reaction might be

*There is a distinct tendency for certain strains to degenerate to the rough type. The intermediate type (SR) gives rise to both rough and smooth daughter colonies but quite often the tendency to degenerate is so pronounced that it is impossible to separate the daughter races without finally losing the smooth forms. For this reason, an effort is made to obtain pure cultures as rapidly as possible and to ignore any minimal dissociation rather than attempt to purify the strain at the possible expense of the loss of some of its pathogenic properties.

of differential value, it was applied to all smooth strains of streptococci isolated from suspected foci of infection. The following method was used for the agglutination tests:

Pure substrains of streptococci in Rosenow's brain heart infusion are washed twice with 1.0 per cent phenol in normal saline and resuspended in the latter in concentrations of about 50 billions per c.c. The concentrated suspensions are diluted with normal saline, allowed to stand for one hour to permit settling of the coarser particles, and decanted. If allowed to stand for a longer period, there is a tendency for the suspensions to give weaker agglutination. Even the concentrated suspensions may lose some of their agglutinability on standing. If the tubes are not decanted, the control tubes will often contain a deposit of bacteria and extraneous matter which is difficult to distinguish from specifically agglutinated particles. With the above precaution, however, the controls show only a minimum of sediment. A special technic is necessary for hemolytic streptococci on account of the autoagglutinative properties of the group. We have used methods described by Spicer,[9, 10] and Mueller and Klise.[11]

Into a series of test tubes is added 1.0 c.c. of serum dilutions ranging from 1:40 to 1:10,240. A saline control is included. To each tube is added 1.0 c.c. of the suspension to be tested. The tubes are shaken gently for two minutes and placed in a water-bath at 50 to 52° C. for two hours. They are allowed to stand overnight at room temperature and the results read the following day. It was found that some of the tubes gave an increased titer when reexamined an hour or so later. This phenomenon was found to be due to the jarring of the tubes consequent to dropping them back into the racks after making the initial observations. Therefore, the tubes are lifted about 1 cm. and permitted to drop against the metal bottoms of the racks. This jarring is sufficient to cause sedimentation of the unstable particles which can be readily observed if the tubes are allowed to stand for one or two hours. After that time the jarring process is repeated and the tubes examined. The examination of the tubes requires critical illumination. This can be improved by placing a black card behind the sediment. The control must always be critically compared with the tubes containing serum dilutions.

Each strain was tested with the serum of the patient from whom it was isolated. In the early work strains agglutinating to a titer of 1:160 or over were used for the preparation of vaccines for the treatment of patients with rheumatoid arthritis. Inagglutinable strains were discarded. While the response to vaccine treatment was favorable in many cases, certain patients were not benefited in spite of the fact that the strains which had been used in the preparation of the vaccines had been agglutinated to a high titer by the patient's serum. This suggested that the agglutination reaction was not sufficiently reliable as a basis for the selection of strains for the preparation of vaccines, possibly because some etiologic strains were inagglutinable or because the serum of certain patients did not contain agglutinins for streptococci.

The possibility of the occurrence of inagglutinable pathogenic strains is not a new conception. It is well known, e.g., that freshly isolated strains of typhoid bacilli are sometimes inagglutinable. More convincing proof of the existence of inagglutinable pathogenic strains of certain bacteria is found in the work of Gilbert and Dacey[12] who isolated *Br. abortus* from the blood clot of a patient whose serum did not contain agglutinins for *Br. abortus*. Certain strains of *Strep. viridans* isolated by one of us (G. H. C.) from blood cultures of patients with long-standing rheumatoid arthritis were not agglutinated by the patient's serum.

In view of the apparent failure of the agglutination reaction for differential purposes, other tests were sought.

Among the serologic tests which have been applied to this type of streptococcus is one which is based on the ability of a strain to grow in or survive exposure to fresh blood. Hare[13] pointed out that the ability to multiply in human blood might well be employed to assess the importance of strains isolated from the nose and throat.

Solis-Cohen and Boerner[14] believed that it was necessary to use the patient's own blood, but Hare[13] tested organisms against normal blood and the blood of the patient and concluded that, in general, there was no difference. Fleming[15] was of the same opinion.

For certain species of bacteria, the susceptibility to the "bactericidal" activity of normal blood is related to the smoothness or roughness of the strain. This was demonstrated by a number of investigators. Fothergill and Wright[17] showed that the virulent smooth influenza bacillus was completely resistant to the "bactericidal" action of diluted, normal, unheated serum, while the rough organism was easily killed when subjected to similar action. Similar conclusions were reached by Pittman.[18] Grinnell[19] found that a 1:12 dilution of blood killed from 2 to 1,200 of the smooth strains of *B. typhosus* and 1,200,000 of the rough type. This difference was reemphasized by him in a study of the commercial Rawlins strains.[20] Wright and Ward,[16] Fothergill and Wright,[17] Rake,[21] Hare,[22] Todd,[23] and Downie[24] proved that the ability of a strain to grow in fresh blood was correlated with its "virulence" for laboratory animals.

The use of a counted number of live bacteria is a ponderous task and, to eliminate this step, the "bactericidal" action was determined by comparing the density of growths obtained from transplants before and after exposure to the diluted blood. As suggested by Gordon and Wormall,[25] cultures of streptococci were mixed with fresh guinea pig blood which had been diluted 1:10, tested for viability, incubated for six hours and retested for viability. The results were difficult to interpret, and there was no significant difference between the growths obtained before and after incubation. While the six-hour growths were often lighter than the initial growths, identical results were obtained by substituting heated blood for the fresh blood. This indicated that the poorer growths were not due to some factor peculiar to fresh blood and, therefore, not to be "bactericidal" principle.

In order to accentuate the effect of the "bactericidal" action, the technic was modified. Fresh, defibrinated, guinea pig blood was diluted 1:4 with sterile saline. Intensification of the "bactericidal" action was still further accomplished by the use of a longer incubation period. However, this rendered the initial mixture unsuitable for control purposes, because it was necessary for the growth obtained from transplants from the initial mixture to stand a day or so until the growth from the final mixture was obtained for comparison. It was considered that, if the active principle was heat labile, the bactericidal action could be estimated by testing the culture simultaneously with heated blood. When a series of strains was tested with 1:4 dilutions of blood, the maximum difference between the growths from heated and unheated blood was found to occur at the end of forty-eight hours.

The technic finally adopted was as follows: Blood was aseptically obtained by heart puncture of a number of guinea pigs, defibrinated with glass beads and pooled. It was then divided into two portions and one of them was heated to 50° C. for thirty minutes to inactivate complement. Higher temperatures, such as are used for the inactivation of hemolytic complement, tended to hemolyze and clot the whole blood. Portions of the heated and unheated blood were separately diluted with three volumes of sterile saline. Dilution of the defibrinated blood with nutrient media, instead of with saline, confused the readings. Two rows of sterile plugged tubes were placed in a rack. To each tube in the front row was added 0.5 c.c. of the diluted unheated blood. The tubes in the rear row received 0.5 c.c. of diluted heated blood. The latter served as controls. A loopful of each overnight culture in brain heart infusion, obtained as previously described, was added to each of a pair of tubes, consisting of a tube of unheated blood and a heated blood control tube. The tubes were thoroughly shaken and incubated for forty-eight hours. After shaking, a loopful from each tube was streaked on blood agar in such a way that the growths from transplants from each pair of tubes could be compared. After overnight incubation, the growths from the fresh blood were compared with growths from the control tubes.

If the growths from the heated and the unheated blood were similar, the reactions were recorded as ++++; if the growth from the fresh blood was slightly diminished, the reaction was recorded as +++; a poor growth from the fresh blood as contrasted with a good growth from the heated blood was recorded as ++ or +; while the absence of growth from the fresh blood as compared with a good growth from the heated blood indicated a negative reaction.

This test, hereafter referred to as the "bactericidin" reaction, was applied to all smooth strains of streptococci isolated, as previously described, from the suspected foci of infection of patients with rheumatoid arthritis.

It is not intended to consider the term "bactericidin" as implying a conviction that the active principle of fresh blood has a "bactericidal" as compared with a "bacteriostatic" action, but until this question has been settled, customary usage will be followed and the term "bactericidin" used to denote the active principle of fresh blood. Organisms highly resistant to the "bactericidal" action (++++ or +++ reactions) are referred to as "bactericidin

positive," while those which are susceptible to the "bactericidal" action (++ or negative reactions) are referred to as "bactericidin negative" strains. The resistant strains are considered "positive" because they appear to be more significant than strains which are sensitive to bactericidin.

Strains which resisted bactericidin were compared with strains which were agglutinated by the patient's serum by injecting live cultures into rabbits. In certain instances more than one type of strain (in regard to the agglutination and bactericidin tests) was obtained from a single patient. These strains were also compared by animal experimentation. The strains selected for study were either: (1) inagglutinable but resistant to bactericidin; (2) agglutinable but sensitive to bactericidin; (3) reacting positively to both tests; or (4) reacting negatively to both tests.

They were grouped as follows:

| GROUP | REACTION | | NO. OF STRAINS TESTED |
|---|---|---|---|
| | AGGL. | BACT. | |
| 1 | 0 | + | 19 |
| 2 | + | 0 | 10 |
| 3 | + | + | 7 |
| 4 | 0 | 0 | 9 |

Each strain was grown in brain heart infusion overnight. The following day 0.5 to 1.0 c.c. was injected intravenously into a rabbit. Injections were repeated about every four days and were increased as tolerated. In those instances where the rabbits showed marked symptoms after the injection (such as loss of weight, loss of appetite, listlessness, stiffness, etc.), the interval was lengthened and the dose reduced until these symptoms were reduced to a minimum. The procedure is somewhat different from that employed by Hadjopoulos and Burbank[26] who used doses of 1.0 c.c. of a twenty-four-hour broth culture daily for four consecutive days.

Injections were continued until the rabbits showed evidence of chronic arthritis. In some cases this required only five injections, in others as many as 20 or more were needed. Joint symptoms usually appeared after the fifth or sixth injection, but in one instance they were not noted until after the seventeenth injection. The rabbits frequently developed diarrhea, listlessness, anorexia, and loss of weight. There was usually a period of acute arthritis of varying duration developing from about seven to ten days after the initial injection, following which the rabbit appeared normal. The development of acute arthritis was not considered of significance. Rabbits were classed as having arthritis when the disease progressed to the chronic stage with deformities and permanent joint disease confirmed by roentgen ray examination.

Roentgen ray studies were made in over 80 per cent of the rabbits. In some instances they showed only a haziness of the joint with swelling of the soft tissues, indicating an early lesion. In others there was absorption of the cartilage with proliferation of the bone, which varied in degree in different rabbits. A diagnosis of arthritis was made only when these reactions were pronounced. In some instances, there was complete disintegration of the

joint. Bowing of the long bones was frequently observed, but this was not considered of significance as it is a normal occurrence in certain rabbits.

Autopsies were made of certain animals in each group and histologic examination of discs of ground bone was made of representative joints from rabbits of each group. In general, all the bone discs showed varying degrees of decalcified bone.

Bone discs were also made from different sections of all the larger bones from certain rabbits with arthritis. All the discs showed evidence of varying degrees of repair of preexisting lesions. These changes were interpreted as evidence that the bones had been affected, not only at the joints, but also throughout their entire length. Sections of the synovial membranes revealed chronic synovitis.

Agglutination reactions with the offending strain were made at irregular intervals in forty-six of the rabbits which developed arthritis. The reactions were positive, i.e., the titer was 1:160 or over, in thirty-eight rabbits (82.6 per cent).

The following summaries illustrate the effects produced by the different types of streptococci:

*Group I Strains.*—In this group there were nineteen strains which were not agglutinated by the blood serum of the patient from whom they were obtained but were resistant to bactericidin. They were injected into rabbits in graded amounts as previously described. Three strains were injected into each of two rabbits to compare their ability to produce arthritis in different rabbits. The rabbits were more severely ill, and more frequently developed diarrhea, lost weight and died from the effects of smaller doses of culture than those rabbits which were injected with strains of the other groups. Four rabbits died during the course of the injections and are not included in the tabulations. All the remaining rabbits developed arthritis and, in almost every instance, it appeared more quickly and was induced by smaller doses than were required for the production of chronic arthritis by other groups of strains. The strains of this group produced arthritis in from 4 to 17 injections, the maximum amount being from 0.5 to 12.0 c.c. Arthritis developed in from seven to seventy-four days after the initial injections.

*Group II Strains.*—This group consisted of 10 strains which were agglutinated by the serum of the patient from whom they were obtained but were sensitive to bactericidin. Graded amounts of cultures were injected into ten rabbits as previously described. Two of the rabbits died during the course of the injections and are not included in the tables. Arthritis developed in seven rabbits (87.5 per cent). After its development they were kept for a prolonged period and several of them died, presumably from arthritis because they showed deformities, marked loss of weight and loss of appetite. Death was apparently due to exhaustion.

In two instances, the strain was injected into each of two rabbits for comparative purposes. Essentially similar characteristics were noted in both rabbits of each pair.

The rabbits inoculated with this group of strains required from nine to

sixteen injections, the maximum amounts ranging from 1.0 to 20.0 c.c. Arthritis developed in from nine to one hundred and eleven days.

*Group III Strains.*—The seven strains of this group were agglutinated by the blood serum of the patients from whom they were obtained and resisted bactericidin. Most of the strains were injected into more than one rabbit for comparative purposes. One of them died during the course of the injections. Arthritis developed in eleven rabbits (91.66 per cent). The morbidity, maximum dose required (0.5 to 10.0 c.c.) and total amount used (50 to 104 c.c.) for the production of chronic arthritis with this group of strains did not differ appreciably from those used of inagglutinable strains which resisted bactericidin. However, symptoms of arthritis did not appear until from thirteen to eighty days, which was somewhat longer than was required for the inagglutinable strains which resisted bactericidin. From four to sixteen injections were required for the production of arthritis in rabbits with this

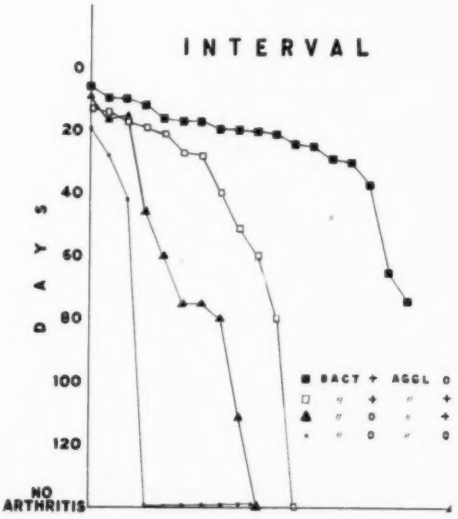


Chart 1.—Comparison of intervals between initial injection and the appearance of chronic arthritis in rabbits injected intravenously with strains of *Streptococcus viridans* which differ in their reactions to the agglutination and "bactericidin" tests.

group of strains. When strains were injected into more than one rabbit, no essential difference was noted except that arthritis was not produced in one rabbit by a strain which had produced arthritis in two other rabbits.

*Group IV Strains.*—This group consisted of nine strains which were not agglutinated by the serum of the patients from whom they were isolated and which were sensitive to bactericidin. The cultures were injected intravenously into rabbits as previously described. Arthritis developed in only three (30 per cent). The interval required for its development (nineteen to forty-two days), the number of injections required (11 to 15), the total amount used (85 to 105 c.c.), and the maximum dose (10 to 12 c.c.) were more than was necessary for the production of chronic arthritis with the other groups of streptococci. When one strain was injected into each of two rabbits, there was no essential difference in its arthritis-producing ability.

Charts 1, 2 and 3 illustrate the differences in the characteristics of the arthritis-producing ability of the four groups of strains. Chart 1 illustrates the interval between the first injection and the development of chronic arthritis. Each rabbit injected with Group I strains developed chronic arthritis in a shorter period of time than rabbits injected with strains of the other groups. Group III strains required longer periods of time than Group I strains which differed from them only by their agglutinability. Strains which were sensitive to bactericidin required still longer periods, particularly if the agglutination reaction was negative.

When strains which resist bactericidin are compared on the basis of their agglutination reactions, it would be expected that agglutinable strains would produce arthritis in rabbits in a shorter period of time than inagglutinable

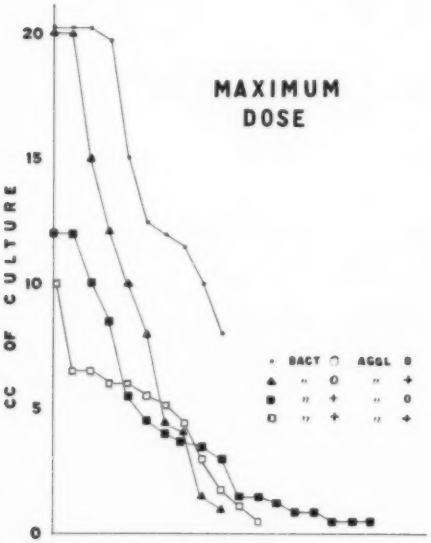


Chart 2.

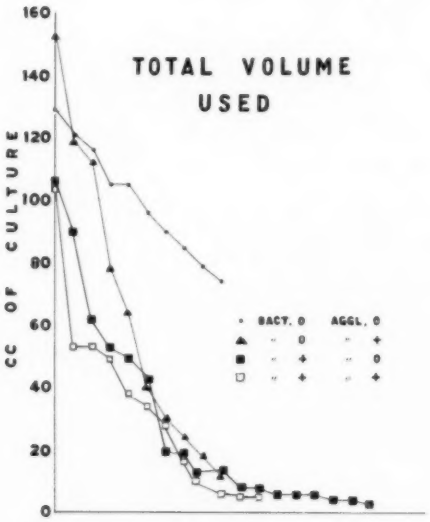


Chart 3.

Chart 2.—Comparison of the maximum doses used for the production of chronic arthritis by the intravenous injection of strains of *Streptococcus viridans* which differ in their reactions to the agglutination and "bactericidin" tests.

Chart 3.—Comparison of total volumes of culture required for the production of chronic arthritis in rabbits by strains of *Streptococcus viridans* which differ in their reactions to the agglutination and "bactericidin" tests.

strains. However, in the series of cultures tested, the inagglutinable strains which resisted bactericidin produced chronic arthritis in a much shorter time than the agglutinable strains which resisted bactericidin.

The maximum dose was fairly characteristic for each of the four groups of strains and was distributed in the following order:

| AMOUNT REQUIRED | BACTERICIDIN REACTION | AGGLUTINATION REACTION |
|---|---|---|
| Highest maximum dose | 0 | 0 |
| Second largest maximum dose | 0 | + |
| Third largest maximum does | + | + |
| Least maximum dose | + | 0 |

The total volumes of culture required for the production of arthritis in rabbits with the different groups did not show a marked difference (Chart 3), but the same sequence was noted as with the maximum dose. A considerably larger amount of culture was used when the strains reacted negatively to both tests. In this case, the larger total volume of culture required was characteristic and was considerably larger than that used for the other groups of strains.

The difference in the rate and severity of infection induced by those strains which resisted bactericidin as compared with those which were sensitive to it is well illustrated by a comparison of the following typical experiments (Tables I and II).

TABLE I

PROTOCOL OF RABBIT INJECTED INTRAVENOUSLY WITH *Streptococcus viridans* STRAIN 341 (BACTERICIDIN REACTION ++++. AGGLUTININ TITER LESS THAN 1:160)

| DATE | AMOUNT INJECTED | OBSERVATIONS |
|---|---|---|
| April 17 | 0.5 c.c. | |
| 18 | 1.0 | |
| 19 | 1.5 | Diarrhea. Stiffness in both hind legs, mostly in hips. |
| 20 | 2.0 | |
| 21 | 2.0 | Definite stiffness in right hip and knee. Listless, appetite poor. |
| 24 | 3.0 | Stiffness slowly decreased from this time. |
| 28 | 4.0 | |
| May 2 | 5.0 | Stiffness disappeared. |
| 4 | 6.5 | |
| 11 | 7.5 | |
| 18 | 8.5* | Left knee swollen and tender. Decided limp. |
| Injections discontinued | | |
| May 24 | – | Carried left leg when moving. Some stiffness in spine. |
| June – | – | No change in leg. Stiffness of spine gradually increased. |
| June 28 | – | Marked deformity of left rear leg. Marked stiffness of spine with head fixed to one side. Chloroformed. Roentgenograms made. Autopsy. |

*Chronic arthritis considered as beginning at this time.

Strain 341 was resistant to bactericidin. It was injected into a rabbit as previously described. Diarrhea developed after the third injection (1.5 c.c.), and acute arthritis was noted after the fifth injection (2.0 c.c.). After the eleventh injection (8.5 c.c.), which was given one month after the first, the left knee was swollen and tender, and there was a decided limp. The injections were discontinued but arthritis persisted until the rabbit was killed forty-one days later.

Strain 8012 was sensitive to bactericidin but was agglutinated to a titer of 1:20,480 by the blood serum of the patient from whom it was isolated. It was inoculated into a rabbit as previously described. The initial dose was 0.5 c.c. This was rapidly increased until the seventh dose (6.5 c.c.) was reached in twenty-one days. The rabbit was listless and there was some generalized stiffness which disappeared thirteen days later. When the dose of 15.0 c.c. was reached (nineteen days after the disappearance of the acute symptoms), there was a slight stiffness and slight tenderness in the right knee. However, definite stiffness was not noticeable until after the fifteenth injection (20.0 c.c.) which was given twenty-three days later.

The results are summarized in Table III.

In an attempt to compare the effect of different types of strains from each of several patients, 23 strains representing 10 groups of 2 or 3 strains were injected into rabbits. Each group was isolated from a single patient. As

TABLE II

PROTOCOL OF RABBIT INJECTED INTRAVENOUSLY WITH *Streptococcus Viridans* STRAIN 8012 (BACTERICIDIN REACTION NEGATIVE, AGGLUTININ TITER 1:20,480)

| DATE | AMOUNT INJECTED | OBSERVATIONS |
|---|---|---|
| December 12 | 0.5 c.c. | |
| 13 | 1.0 | |
| 14 | 1.5 | |
| 15 | 3.0 | |
| 19 | 4.0 | |
| 26 | 5.0 | |
| January 3 | 6.5 | Listless. Some generalized stiffness. |
| 10 | 7.0 | |
| 16 | 8.0 | Stiffness has disappeared. |
| 23 | 10.0 | |
| 30 | 12.0 | |
| February 4 | 15.0 | Slight stiffness in right knee. Slight tenderness. |
| 14 | 18.0 | |
| 20 | 19.0 | |
| 27 | 20.0* | Definite stiffness in right knee and right hip. |
| March 5 | 20.0 | |
| Injections discontinued | | |
| March 26 | – | Carried right leg when moving and favored left fore paw. Stiffness gradually increased in right rear leg and left fore paw. The left rear leg also became involved. Extremely crippled and moved with difficulty. |
| July 10 | – | Chloroformed. Roentgenograms made. Autopsy. |

*Chronic arthritis considered as beginning at this time.

TABLE III

| GROUP | BACTERICIDIN | AGGLUTININ | MEAN INTERVAL (IN DAYS)* | MEAN MAXIMUM DOSE (IN C.C.) | MEAN TOTAL VOLUME USED (IN C.C.) | MEAN NO. OF INJECTIONS |
|---|---|---|---|---|---|---|
| 1 | + | 0 | 25.0 | 4.1 | 28.2 | 9.9 |
| 2 | 0 | + | 54.0 | 9.6 | 60.8 | 13.1 |
| 3 | + | + | 33.7 | 4.7 | 34.0 | 11.9 |
| 4 | 0 | 0 | 30.0† | 14.9 | 99.8 | 13.7 |

*Before the development of arthritis.
†On the basis of only three strains.

previously stated, the object of studying this group was to determine if strains isolated from the same patient but differing in their reactions to the two in vitro tests differed in their ability to produce arthritis in rabbits. Three rabbits dying after the third or fourth injection and one rabbit dying following an injury are not included in the table (Table IV).

The production of arthritis in rabbits by the injection of strains of these groups was apparently correlated with the serologic type of the strain. In general, if the strain resisted bactericidin, arthritis was produced in a shorter

time, a smaller number of injections were required, and a smaller maximum dose was necessary than was the case with strains which were sensitive to bactericidin.

TABLE IV

COMPARISON OF THE EFFECTS OF THE INTRAVENOUS INJECTION OF RABBITS WITH DIFFERENTLY REACTING STRAINS OF STREPTOCOCCUS VIRIDANS FROM THE SAME PATIENT

| PATIENT | STRAIN NUMBER | BACTERICIDIN REACTION | AGGLUTININ TITER | INTERVAL (DAYS)* | MAXIMUM DOSE (C.C.) | TOTAL AMOUNT (C.C.)† |
|---|---|---|---|---|---|---|
| F. M. | 8963 | ++ | 1:10,240 | 50 | 6.0 | 52.5 |
| | 8965 | ++++ | 1:640 | 28 | 1.8 | 4.5 |
| M. J. | 343 | 0 | 1:640 | 13 | 4.5 | 29.3 |
| | 341 | ++++ | 0 | 37 | 8.5 | 49.5 |
| | 339 | ++++ | 0 | 15 | 3.5 | 11.0 |
| R. T. | 719 | ++++ | 1:640 | 10 | 0.5 | 5.0 |
| | 712 | +++ | 0 | 14 | 1.5 | 12.2 |
| A. S. | 9972 | ++++ | 0 | 17 | 0.5 | 6.5 |
| | 9973 | 0 | 1:640 | 7 | 8.0 | 55.6 |
| C. K. | 8177 | +++ | 1:320 | 30 | 5.5 | 16.7 |
| | 7842 | ++++ | 0 | 24 | 4.5 | 19.2 |
| L. D. | 708 | 0 | 1:640 | 15 | 1.5 | 16.0 |
| | 744 | +++ | 0 | 10 | 0.5 | 3.5 |
| W. H. | 502 | ++++ | 0 | 30 | 4.0 | 53.6 |
| | 543 | ++++ | 1:640 | 10 | 5.0 | 39.3 |
| T. H. | 703 | ++++ | 0 | 17 | 0.5 | 2.5 |
| | 704 | ++++ | 1:640 | 5 | 1.5 | 7.8 |
| | 750 | 0 | 1:1,280 | 6 | 1.5 | 13.7 |
| E. K. | 528 | ++++ | 0 | 2 | 3.0 | 13.4 |
| | 521 | +++ | 1:160 | 7 | 4.0 | 19.5 |
| | 523 | 0 | 1:640 | 3 | 1.0 | 10.0 |
| M. L. | 9536 | ++++ | 0 | 76 | 12.0 | 10.5 |
| | 9523 | ++++ | 1:1,280 | 20 | 12.0 | 87.1 |

*Interval before the appearance of chronic joint symptoms.
†Total amount of culture used.

## SUMMARY

Methods are described for the selective isolation of smooth strains of streptococci from suspected foci of infection of patients with chronic rheumatoid arthritis. When isolated, these strains were tested for agglutinability by the serum of the patients from whom they were isolated. Because vaccines prepared from certain strains with high agglutinin titers produced no therapeutic response when injected into patients with chronic rheumatoid arthritis, it was suggested that these agglutinable strains did not possess immunogenic value or that strains of etiologic significance may not have been agglutinable by the patient's serum.

From this it was reasoned that other factors might be involved in the antigenic structure of streptococci isolated from patients with rheumatoid arthritis. It seemed possible that some inagglutinable strains might contain

important factors which were not present in the agglutinable strains under consideration. Attempts were made to differentiate streptococci by other in vitro tests and to compare the reactions to these tests with the ability of the strain to produce chronic rheumatoid arthritis in rabbits.

One of the reactions studied was based on the "bactericidal" action of normal blood and was applied to a number of strains of streptococci. Strains were differentiated on the basis of their reactions to this "bactericidin" test and their agglutinability, in titers of 1:160 or over, with the serum of the patient from whom they were obtained. Those strains which reacted to only one of these tests were selected for animal inoculation. The respective abilities of these strains to produce arthritis in rabbits were determined by the intravenous injection of live cultures in doses which were progressively increased until chronic arthritis developed or until the volume injected each time reached 15 to 20 c.c. Strains reacting positively or negatively to both tests were used for comparison with strains reacting positively to only one test. In addition, ten groups of strains, each containing different types of strains isolated from a single patient, were similarly injected into rabbits to determine if there were differences in the arthritis-producing ability of the various strains which are closely associated in a suspected focus of infection. Certain strains were injected into more than one rabbit for comparative purposes.

Chronic arthritis was produced in 90 to 100 per cent of rabbits by strains which either resisted the "bactericidal" action of fresh, diluted, defibrinated guinea pig blood or were agglutinable by the patient's serum. However, it was produced in only 30 per cent of the rabbits by strains which did not resist the "bactericidal" action of fresh, diluted, defibrinated guinea pig blood and which were inagglutinable by the serum of the patient from whom they were obtained.

In those instances where several strains differing in their reactions to these two tests were isolated from the same patient and injected into rabbits, arthritis was produced in rabbits by the strains which were either agglutinable, in a dilution of 1:160 or over of the patient's serum, or which resisted the "bactericidal" action of fresh, diluted, defibrinated guinea pig blood; but the rate of development differed considerably and was correlated with the reaction of the strain to the agglutination and "bactericidin" tests.

When all the different groups are considered, strains which reacted only to the bactericidin test produced arthritis in rabbits in a shorter period of time and required fewer injections, a smaller maximum dose, and a smaller total volume of culture than strains of any other group.

Next in order were those strains which, in addition to their positive reactions to the bactericidin test, were agglutinable by the serum of the patient from whom they were isolated.

When the agglutination reaction alone was positive, the strains required a longer interval, a larger maximum dose, a larger total volume of culture, and more injections than the two previous groups.

Finally, strains which reacted negatively to both the bactericidin and agglutinin tests took the longest time for the production of arthritis in rabbits, required the largest number of injections, the largest maximum dose, and the largest total volume of culture of any group of strains.

While consideration has been given only to the relationship between the agglutination and "bactericidin" reactions of streptococci and the ability of the strains to produce chronic arthritis in rabbits, it is possible that these factors may also be associated with other pathogenic factors. For example, there was evidence that those strains which resisted the "bactericidal" action of diluted, fresh, defibrinated guinea pig blood were more toxic for rabbits than those strains which were sensitive to the "bactericidal" action. No such relationship could be demonstrated between the agglutination reaction and toxicity for rabbits.

## CONCLUSIONS

Strains of streptococci which are resistant to the "bactericidal" action of fresh, diluted, defibrinated guinea pig blood are capable of producing chronic arthritis in rabbits. Strains which are not resistant to the "bactericidal" action but which are agglutinable by the serum of the patient from whom they were obtained are also capable of producing chronic arthritis in rabbits, but require larger doses of culture, a longer period of time, and a larger number of injections than those strains which are resistant to the "bactericidal" action. Strains which do not respond to either the "bactericidal" or the agglutinin test are often incapable of producing chronic arthritis in rabbits under the conditions used in these experiments.

Therefore, (1) the reaction of a strain to the "bactericidal" action of fresh, diluted, defibrinated guinea pig blood, and (2) the agglutination reaction with the patient's own serum are of value for the study of streptococci isolated from suspected foci of infection of patients with chronic rheumatoid arthritis.

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# *LABORATORY METHODS*

## THE EFFECT OF HYDROGEN ION CONCENTRATION UPON THE DETERMINATION OF CALCIUM IN BLOOD SERUM PHOSPHOMOLYBDIC ACID CENTRIFUGATES*

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VON BERENCSY[1] has reported a chemical method for the quantitative determination of nonprotein bound calcium in blood serum, in which the protein is precipitated with phosphomolybdic acid and the filtrate analyzed for calcium. He found 60 to 65 per cent of the calcium in the filtrate, and he assumed that the remainder, which was precipitated along with the protein, was actually bound to the latter. In order to obtain sufficient material from small amounts of rabbit's blood, Hermann[2] modified the method by centrifuging, instead of filtering off the precipitate resulting from the addition of 4 c.c. of 2.5 per cent phosphomolybdic acid to 2 c.c. of serum.

In order to test the assumption of von Berenesy and Hermann that the calcium precipitated with the protein was actually bound to it, Rosenthal[3] added increments of a calcium chloride solution to a phosphomolybdic acid serum mixture, and was unable to recover the added calcium quantitatively in either centrifugate or filtrate. He therefore concluded that protein precipitation by phosphomolybdic acid, followed by calcium determinations in the filtrate or centrifugate and total calcium determinations on the serum, gives no information as to what part of the serum calcium is in the bound or the unbound form.

In the course of a pharmacologic investigation on the blood serum calcium of cats and dogs, the von Berenesy-Hermann method was given a trial for the determination of nonprotein bound calcium. It was observed, however, that the appearance of the centrifugates obtained from the addition of two parts of 2.5 per cent phosphomolybdic acid to one part of serum varied with individual animals of the same species, in that some were clear, while others were opalescent or cloudy, indicating incomplete precipitation of protein, which was demonstrated to be true upon the addition of more acid to the suspected centrifugates. Calcium determinations on the oxalate precipitates of these centrifugates yielded very confusing results, and it was decided, therefore, to investigate the relationship of hydrogen ion concentration to calcium content in serum phosphomolybdic acid centrifugates.

Various studies were conducted on blood serum. In this paper the results obtained with one cat and two dogs are reported. The cat was anesthetized with

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sodium barbital and the dogs with nembutal. The animals were bled by cannula from the carotid artery. Serum and phosphomolybdic acid were mixed in various proportions, allowed to stand about half an hour, and thoroughly centrifuged. The hydrogen ion concentration of the decanted centrifugates was determined with the quinhydrone electrode, and their calcium content, as well as that of the trichloracetic acid centrifugate for total calcium, was determined by the Clark and Collip[4] modification of the Kramer-Tisdall method.

The results of these studies are shown in Fig. 1. All centrifugates with a hydrogen ion concentration on the alkaline side of approximately pH 4.1 were opalescent or cloudy, insufficient acid being present to cause complete protein precipitation. Some of the suspended protein came down with the oxalate precipitate, so that in titrating, permanganate was reduced by protein as well as

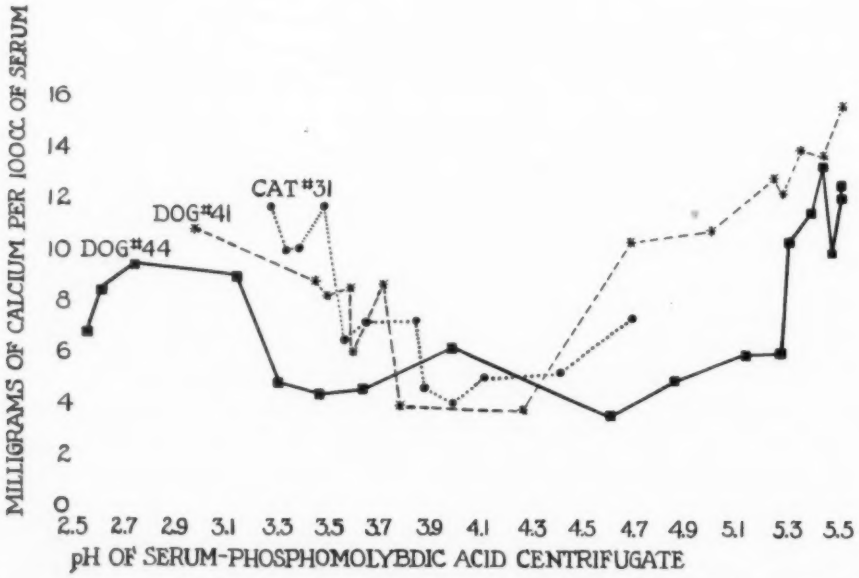


Fig. 1.—The relation of pH to calcium recoveries in serum phosphomolybdic acid centrifugates. (Total Calcium Values: Cat 31, 11.9; Dog 41, 12.2; and Dog 44, 12.1 mg. per 100 c.c. of serum.)

by the oxalates of protein bound and nonprotein bound calcium. As a result, calcium calculations based on the amount of permanganate reduced were too high, the values in some cases being greater than the amount obtained in the separate analysis for total calcium. On the other hand, all centrifugates with a hydrogen ion concentration on the acid side of pH 4.1 were clear, indicating that there was also an irregular but progressive increase in calcium, due to removal of calcium from protein by excess acidity. Therefore, in those cases where the acidity exceeded the minimum necessary for complete protein precipitation, permanganate titration of the oxalate precipitate measured not only the original nonprotein bound or free calcium, but in addition calcium which had been released from the protein precipitate by the excess acid.

## SUMMARY AND CONCLUSIONS

The addition of two parts of 2.5 per cent phosphomolybdic acid to one part of serum, the procedure used in Hermann's modification of von Berencsy's method for determining unbound calcium, does not always produce the exact hydrogen ion concentration needed for complete protein precipitation. When protein precipitation is incomplete, permanganate titration for calcium yields high values. High values are also obtained for unbound calcium when the acidity is greater than the minimum needed for complete protein precipitation, since this causes the removal of calcium from the precipitate. Since the amount of protein in blood serum is known to vary widely under different conditions,[5, 6] it is obviously impossible to produce a hydrogen ion concentration from the addition of a fixed amount of phosphomolybdic acid that will quantitatively precipitate the serum proteins and at the same time not remove bound calcium from the precipitate. It therefore appears that the von Berencsy-Hermann method, employing a fixed amount of acid, is not satisfactory for the determination of nonprotein bound or free calcium in blood serum.

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# THE TECHNIC OF MOLDING AND CASTING FOR THE MEDICAL SCIENCES*

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*DEFINITION.*—Molding and casting are processes of reproducing an object in form, texture, and sometimes color identical in appearance to the original from which the copy or reproduction is made. To obtain such casts there are two main processes to be undertaken. The first is that of producing the mold, or negative. The second is the making of the cast or positive from the mold. In other words: a mold is an impression of an object such as a face, a hand, a coin; while a cast is an impression of the mold that was made of the object such as a face, a hand, or a coin. In science, the finished cast or positive is sometimes called "moulage," a word of French origin (mouler) meaning to cast, or to mold. The words "negative" and "positive" will also be used in this text as a means of simplification. I prefer the word "moulage" to express both molding and casting as a unit, though the word "casting" is often used alone to express both processes.

*Purpose.*—The object of this text is not to dilate upon the history or application of molding and casting or the moulage process, but to describe practical methods of compounding correct formulas. One might say, however, the growing belief in visual education is a sufficient reason for its intelligent and successful application. Every physician realizes the value of the "before and after" representation of a surgical or medical case. The three dimensional model, naturally colored, leaves little to the imagination and gives practical evidence not apparent in photographs and drawings. The physician and dentist find them well worth while where plastic and oral surgery is done to change the facial contours. They are invaluable in lecture and reference work. They are a permanent and natural means of preserving a case record. By means of photography and photomechanical reproduction for publication, pictures in black and white, or natural colors, of such models can be obtained on paper, loose, or in book form for more convenient preservation and dissemination.

*Compositions for Making the Negative.*—The two main substances used for obtaining the negatives of medical subjects are *plaster of Paris* (gypsum), and a hydrocolloidal composition which we will call "*agar mold*," and "*agar composition*" because its important constituent is agar. Sometimes the *wax mold* is employed. For inanimate and nonprotoplasmic objects, the *glue mold* is often used; and sulphur molds are rarely used. Other substances such as metal, rubber, baked sand, terra cotta, and similar materials are employed for mold making in commercial manufacturing. In medicine there are cases

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in which plaster should be used alone and other cases in which it is more convenient to use agar composition, as this can be easily removed from undercuts. It is not advisable to try to use one substance for all cases. Sometimes plaster is used as a shell to support an agar mold. Plaster of Paris in dry powdered form becomes rigid or *set* when mixed with water. This setting takes place in a relatively short time. If properly used, it will give a negative that has an infinite amount of desirable detail from which the positive is finally made. However, its great disadvantage is the fact that it is not plastic or pliable as is the agar or glue mold.

*Molds in General.*—One of the best descriptions that has been written on the making of facial masks and molds as well as casts of the inside of the

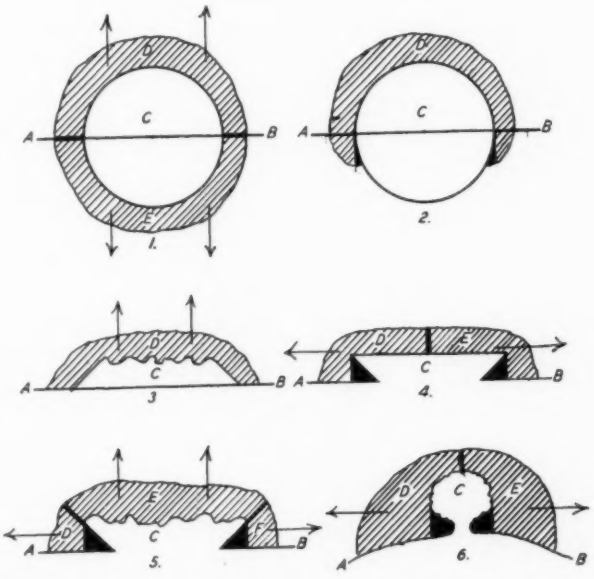


Fig. 1.—Diagrams of molds. The white area represents the rigid subject that is being cast; the shaded area represents the molds and the black areas the undercuts. Arrows point in the directions that the molds, or their pieces, are moved to separate them from the original subject or cast.

mouth appears in *Practical Orthodontia*[1] by Dewey. This author considers no casting materials other than those used in plaster and glue molds with which most casters are familiar.

Many books on modeling and sculpture, such as the ones by Toft[2] and Glass,[3] give interesting points on the technic of producing plaster and glue molds. Much information in the nature of formulas may be found in *Dental Formulary.*[4] A most informative book, from a technical standpoint, is by Frederick,[5] who writes about the making of plaster molds of many kinds. He also gives some description of wax and sulphur molds. Much has been written about plaster molds in hundreds of books. It is for this reason that little will be said about them in this article.

*Undercutting.*—The main difficulty which the caster experiences is that

caused by *undercutting*. An *undercut* is the part of the original subject or mold that is to be cast which prevents the impression from slipping freely away or separating.

For example, let us consider Fig. 1. The white areas, or *C* in these diagrams, represent the subject to be cast. The shaded areas represent the mold and the black areas represent the undercuts. The undercuts or black areas are always a part of the mold and must be considered as such. The arrows denote the directions in which the mold must be pulled to separate it from the subject.

Let us consider these diagrams separately. *C* of Part 1 can represent a sphere of wood or stone. If we wish to cast such a subject, it should be done in two pieces. Such a mold is called a piece mold. The line *AB* is imaginary and passes directly through the center of the sphere, which would divide the mold into two separate pieces, *D* and *E*. Should one-half of this piece mold lap over beyond the imaginary line *AB*, Part 2, then it would be impossible to separate the sphere from a rigid mold without breaking the mold. The black area in Part 2 represents the undercut that would prevent the separation. *C* of Part 3 represents the type of subject that has no undercuts. Therefore, for such an object, it is necessary to make a mold of only one piece. The line *AB* now becomes a flat surface such as a table top upon which the subject rests. In Part 4, consider that the subject is attached to the surface *AB*. However, *C* has a smooth top surface. In such a case a mold of two pieces can be made of a subject providing it is possible to separate the two pieces of mold by pulling them in the opposite directions as indicated by the arrows. It is only necessary to separate them far enough to be relieved of the undercut. In Part 5, we have a subject similar to that in Part 4. However, the top surface is irregular rather than smooth. In such a case it becomes necessary to make a mold of three pieces as the rough surface would prevent drawing a two piece mold to the right and left.

In Part 6 of Fig. 1, let us consider *C* to be a pedunculated tumor attached to the surface of the body *AB*. Such an object can be made in a two piece mold *D* and *E*.

*Sulphur Molds.*—Sulphur molds are unsuitable for use in making impressions of living or dead tissue and are therefore not considered in detail. They are sometimes used for reproducing impressions of coins, medals and medallions, or other delicate reliefs in which there is no undercutting. A wall of thin metal is placed around the subject to be cast and both the subject and wall are shellacked or greased. Eight parts of sulphur and one part of iron filings are melted together and allowed to cool. This mixture while still in a liquid state is poured over the subject, and blown into depressions. When completely cooled, the mold is greased and a plaster impression is made from the sulphur mold.

*Wax Molds.*—Frederick in his book, which was first published in 1899, writes of wax molds as follows: "Molds may be successfully made of wax, usually of small or delicate objects, as fruit, flowers, small animals, coins, medals, or of the hands and face. Take one part of rosin and three parts of

white wax. Melt them in a hot water-bath, as a glass jar placed in boiling water, and heat to the boiling point. Or melt paraffin wax (as a paraffin candle), and add a little sweet oil and whiting."

He advises that the face should be greased as he has previously described (for making plaster molds) and the wax can be applied as soon as it cools to a bearable point. He continues: "Brush it on quickly to a thickness of about ⅛ inch, and, as it almost immediately hardens, an outer shell or case of plaster can be cast before the model feels compelled to move. Remove the case from the wax and the wax from the model, replacing it in the case at once to prevent warping or breaking, and make the cast as soon as possible."

In the June, 1926, issue of *The Journal of the American Dental Association*, Golden[6] describes his method of making wax molds of the face. In a later issue[7] of the same publication, Dr. Golden condenses his first article and discusses other materials in use for the same purpose, such as plaster and "Negocoll." In short, his wax mold method is as follows: A mixture of waxes is prepared which he uses to spray on the patient's face to obtain the mold. This mixture which he terms B-11 contains:

| | | |
|---|---|---|
| Paraffin wax | 55% | (Melt. point 51.7° C.) |
| Bayberry wax | 20% | (Melt. point 40.9° C.) |
| Carnauba wax | 5% | (Melt. point 84.8° C.) |
| Stearic acid | 20% | (Melt. point 69.9° C.) |

Resulting melting point of mixture 52.97° C.

According to the author, no preliminary preparation of the skin surfaces is necessary. However, vaseline can be used on the eyelashes and brows to prevent the hairs from sticking to the mold.

For spraying the wax mixture on the face, the author used a DeVilbiss type CB spray gun. It seems that any spray gun, such as is used for spraying oil paint, should be suitatble for this purpose. The first layer of wax was applied with an air pressure of only four to six pounds. When the air pressure is increased above this point, the deposit becomes uneven and a mist results. This first layer serves as an insulator for a second layer which can be about half an inch in thickness. The latter can be applied with a spatula when the wax is in a congealing state. Little rubber bags filled with ice or dry ice (magic ice) serve well for cooling or chilling the wax mold at different stages of the process. About one inch of wax should be applied, after which the mold is supported with a plaster of Paris shell, or mother mold, as seen in Fig. 2.

This same author gives the following wax composition, which can be painted on the face with a camel's hair brush rather than sprayed:

| | | |
|---|---|---|
| Bayberry wax | 50% | (Melt. point 40.9° C.) |
| Paraffin | 25% | (Melt. point 51.7° C.) |
| Stearic acid | 25% | (Melt. point 69.9° C.) |

Resulting melting point of mixture—41.7°-42.8° C.
(Sayboldt test)

Golden continues: "As this combination continues plastic until possibly thirty degrees above its melting point, it is adaptable for use in brush applications to take impressions and advisable to use for partial facial impressions."

The technic of application is to brush on a thin layer first when the wax is in a liquid state and not hot enough to be uncomfortable for the patient. When the wax reaches the congealing state it can be applied with the spatula to increase the thickness of the mold. The wax is chilled from time to time with the ice pack. It is finally reenforced with a plaster shell.

Golden describes "Negocoll" and quotes from an article by Gruenebaum,[8] which gives no formulas and tells only how to heat and apply "negocoll" and "hominit" which can be obtained from advertising matter on these commercial products or from Poller's book.[9] Golden also repeats some of the same information that may be found in Poller's American Patent,[10] none of which is of specific value in compounding a formula of definite proportions.

Fig. 2.—Diagram of the wax mold process of Mr. Frederick and Dr. Golden. (Redrawn from Dr. Golden's article in the *J. Am. Dent. Assn.*)

In my opinion a hydrocolloidal negative of the face such as an agar mold is more simple to make than a wax mold. Such a mold requires no elaborate equipment and the result should be equally as good if not better. Furthermore, resin-wax compositions, as well as gypsum, "hydrocal," and other positive masses, can be poured, brushed, or otherwise applied into the agar mold, a thing which is impossible with the wax mold. This is due to the fact that the wax mold would melt were the hot positive compositions applied. We will discuss Golden's article further under positive materials.

*Glue or Gelatin Molds.*—The glue or gelatin mold is of comparatively recent origin, probably being less than a hundred years old. Either material will serve well for such a mold. However, the gelatin contains less impurities and is therefore better. Glue is less expensive and for this reason is more in use. Both glue and gelatin can be used over and over again, provided they are properly dried. They were the first molds that were pliable to any great

extent. By their use a great amount of undercutting is taken care of in the stretching or bending of the mold. Glue and gelatin molds have a rubber-like quality of springing back into their original form when once they are removed from the cast. This is accomplished by stretching the negative away from the cast in order to remove it from the mold. In other words, the elastic quality of glue or gelatin allows it to be pulled from beneath projections and around bulging surfaces after which it immediately springs back into its proper position.

Due to the fact that the proper concentration of gelatin or glue and water requires a great amount of heat to bring it to the liquid state for pour-

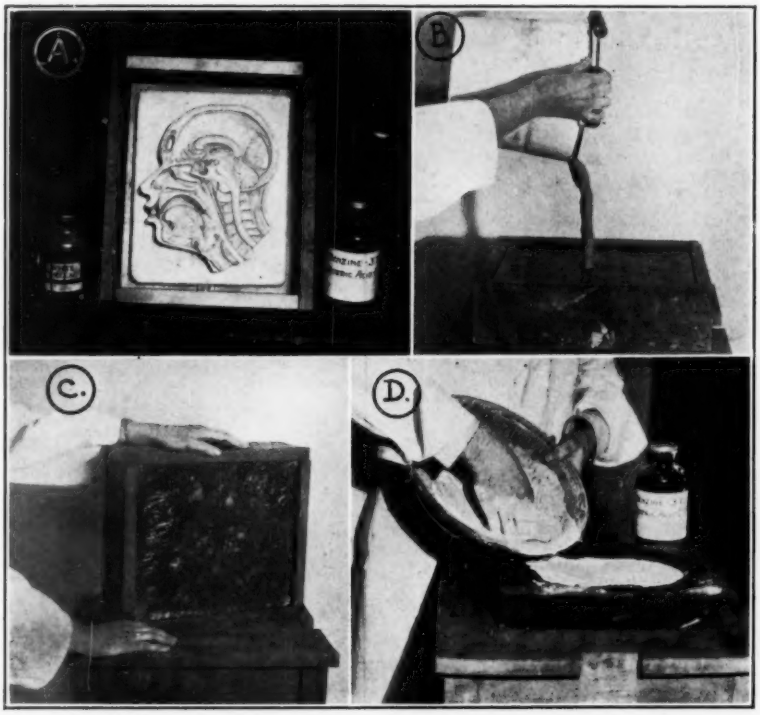


Fig. 3.—The glue mold process. *A*, The plaster subject that is being cast. This is shellacked then painted with a mixture of stearic acid 1 part and benzine 3 parts. *B*, Pouring the hot glue over the plaster cast. *C*, The outside of the mold after the glue has cooled and set. The original cast is removed from the mold. *D*, The mold is painted with the stearic acid-benzine mixture and poured full of plaster. The duplicate cast is removed immediately upon setting; otherwise the heat created by the setting plaster is likely to melt the glue of the mold.

ing, it is, therefore, unsuitable for making a mold directly over living or dead tissue. Furthermore, it takes a considerable time to cool and set, which makes it more unsuitable. In the case where heat is likely to have a destroying effect, it is necessary to use a substance that can be applied in a liquid state, but which will not burn the subject, yet will set, taking the negative form of the tissue upon which it is applied.

The *agar mold* has the qualities of melting at slightly below 100° C. (212° F.). However, it does not return to its semisolid or plastic state until it cools to a temperature of about 42° to 44° C. (108° to 112° F.). Since this is

just about body temperature (37° C. or 98.6° F.), agar, which has many of the other qualities of glue, suggested itself for plastic casting of living subjects. This composition can be applied in a liquid state before it cools to its setting point, without doing harm to living or dead tissue.

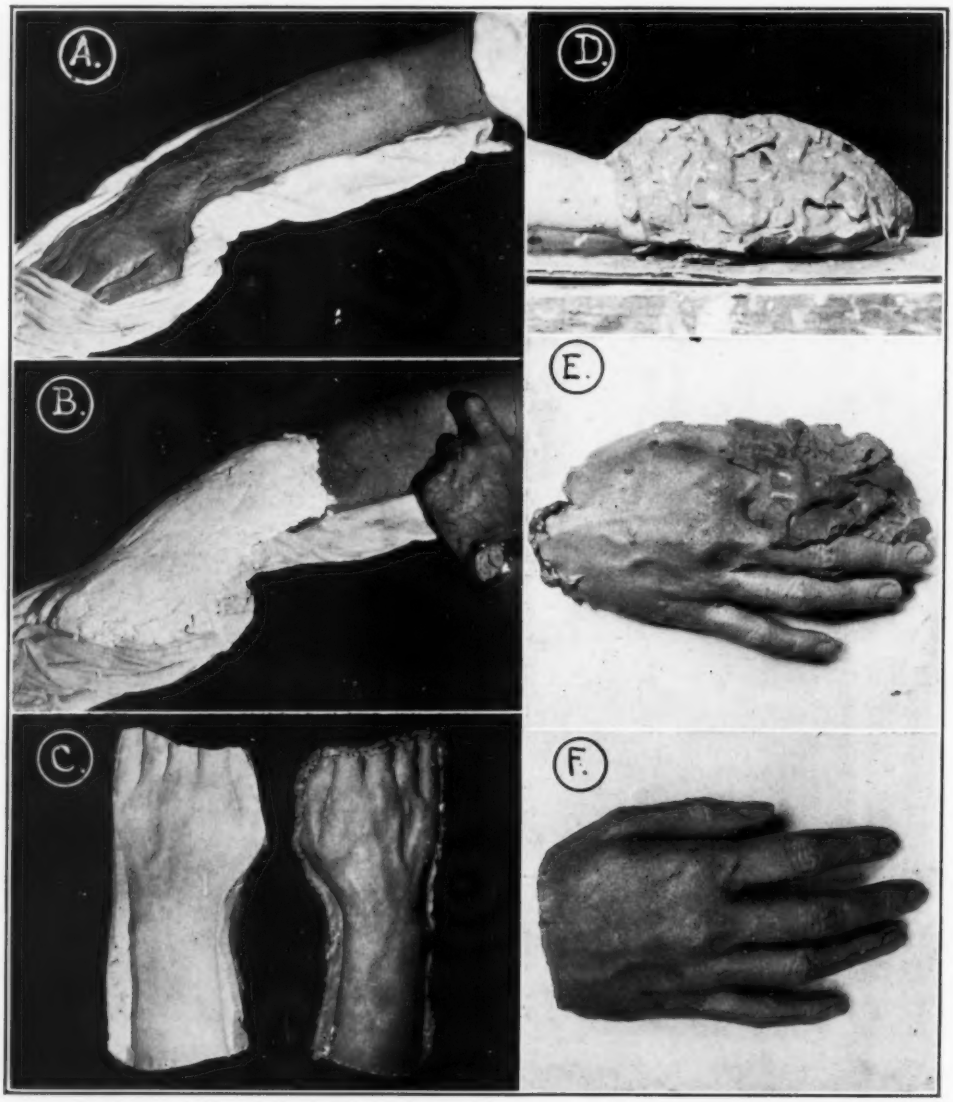


Fig. 4.—Left, simple plaster mold and cast. Right, simple agar mold and cast. *A*, The hand and arm are well greased and placed on a cloth. *B*, They are then covered to a thickness of about a half inch with plaster. A brush is used for the early applications. *C*, The plaster mold is soaked in water then filled with the resin-wax composition. There are no undercuts in such a mold. *D*, Greasing the subject is unnecessary when using agar composition. In this case, the hand is covered both above and below with the composition to a thickness of about one inch. After complete setting the hand is slipped out of the mold as one removes a glove. The mold is then filled with resin-wax composition. *E*, After cooling, the mold is broken away from the cast. *F*, The cast after removal from the mold. Notice the detail.

Agar and water alone are not suitable for plastic casting, as this mixture is too thin to remain on dependent surfaces and tends to fracture or crumble

too easily. It is necessary for this composition to contain other substances such as preservatives, tanning agents, and materials that will increase the colloidal binding power of the agar, causing the particles to hold together and become more rubber-like. This is the important material for making molds of living and dead tissue.

*The Poller System.*—The late Dr. Alphons Poller[9] was evidently the first to recognize the qualities of agar for use in casting and developed his mouage system, after which he wrote a book on the subject.

He evidently intended to commercialize his discovery for his compositions were patented, his trade names of "Negocoll," "Dentocoll" (negative compositions), "Granulit," "Celerit" and "Hominit" (positive compositions) were registered. Nowhere in his book does he divulge the contents of his compositions. A firm in Switzerland has the rights to manufacture his products which are sold in this country at exorbitant prices. In 1933 there

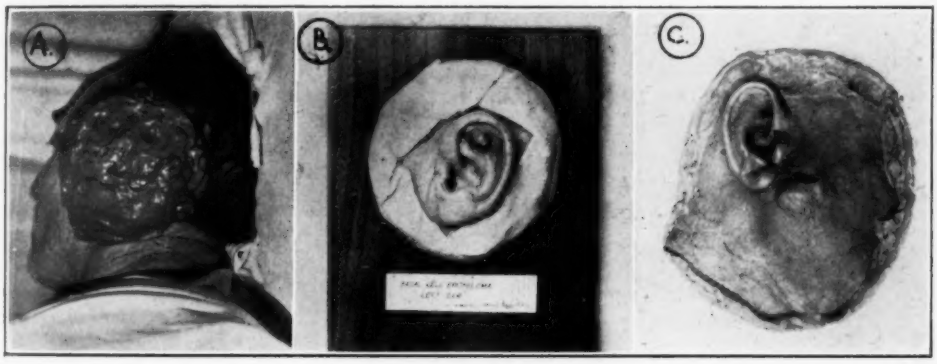


Fig. 5.—Casting an ear. *A*, Small wad of cotton is placed in the deep part of the ear. The agar composition is poured or "painted" on to the thickness of about one inch. *B*, The mold is removed, filled with positive composition, colored, and mounted. *C*, Cast of an ear and tumor of the cheek immediately after removal from the agar mold.

appeared on the market a product called "Form-All" which has the same qualities as negocoll and dentocoll. It is manufactured by an American firm and is materially lower in price than the Poller materials. Their positive material is called "Form-Vex." Another American firm manufactures a negative hydrocolloidal composition which is called "Plastico." The same firm's positive material is known as "Posmoulage."

There are also similar negative compositions called "Surgimould" an elastic compound used by dentists for obtaining impressions of the teeth. In my opinion, all of these molding compositions have an agar base.

In the December issue of *Archives of Pathology*, Volume 16, Number 6, Gross[11] describes a hydrocolloidal composition of his own discovery and gives the following formula and directions for its preparation:

*Preparation of the Negative Mass:* "The negative mass consists of agar,

magnesium soaps, absorbent cellulose wadding, and water in the following proportions:

| MATERIAL | PARTS BY WEIGHT |
|---|---|
| Agar | 100 |
| Oil soap | 100 |
| Magnesium sulphate | 40 |
| Absorbent cellulose waddings* | 12 |
| Water | 700-800 |
| (This is variable: see text) | |

"The agar is heated in about 2,000 c.c. of water until it is completely dissolved. The cellulose wadding is macerated in hot water until the fibers have separated. It is then thoroughly stirred into the hot agar. The oil soap is next added, and when it is completely dissolved and incorporated into the agar mass, a concentrated solution of the magnesium sulphate is slowly poured into it. At the same time, the mass is vigorously stirred. This precipitates the insoluble magnesium soaps. The mass solidifies at about body temperature. The solid material is chopped fine, or, preferably, ground in a food grinder. Since this mass contains from two and one-half to three times too much water, it is spread out thinly on paper and allowed to dry at room temperature to a state in which the crumbs are distinctly moist but no water can be expressed. The material is then ready for use."

In a British patent specifications for Poller[12] is given the following formula:

| | |
|---|---|
| Agar | 1,000 grams |
| Water | 20,000 grams |
| Cellulose | 200 grams |
| Iron Oxide | 500 grams |
| Long fibers | 10 grams |
| Para rubber (not including the weight of the solvent) | 250 grams |

This document continues to explain in a vague way how the material is mixed and the fact that glycerin can be added for exclusively cosmetic purposes.

It also mentioned the fact that colloidal silver was added as a preservative and for its medicinal value.

The above mentioned formula as it now stands has definite disadvantages. I doubt the qualities of colloidal silver as a preservative, and advise the use of other harmless preservatives which are not mentioned in the aforesaid patent specifications. The iron oxide gives the composition a disagreeable red color that can be dispensed with by the substitution of another metallic oxide such as zinc oxide. Agar mixes readily with water over 100° C., quickly liquefying, making prolonged cooking unnecessary as is suggested in the patent. However, these specifications should be of value to the research worker in spite of their complicated form.

Unfortunately the terminology of patents is purposely made complicated and misleading to prevent duplication, and especially commercialization, of the product by unauthorized competitors. It is for this reason that little of

*Cellulose wadding from the so-called sanitary napkins or pads was used.

specific and immediate value can be gained from them. However, they serve well as reference and sometimes give worth-while ideas for experimentation.

While Poller suggests the use of a small amount of wax to raise the setting point in his American patent,[10] I have been unable to obtain the desired results by the use of the quantity of wax suggested. I think it should be eliminated entirely.

It has been my experience to find that such chemicals as metallic sulphates, too much wax or para rubber, have a tendency to reduce the setting point of the negative compositions or to prevent the agar from setting entirely. Poller's composition which he calls "negocoll" is quite good for the purpose of making plastic molds of human tissue, and the author believes it superior to any other agar composition on the market. However, its comparative costliness makes it necessary for the average worker to compound his own agar composition, which at the present time can be done for less than a tenth of the cost of the commercial product.

In preparing a plastic negative composition, the worker must bear in mind that he is making an effort to produce a product that has definite necessary qualities which may be enumerated as follows:

1. It must, in one state, be sufficiently liquid to flow into the smallest cavities.

2. It must be in this same state, viscous or adhesive enough to cling to a vertical plane against the pull of gravity.

3. It must not be harmful or painful to the living or dead tissues of the human body, when it is applied directly to such tissues.

4. It must be of a nature that will change from this liquid state to a solid or gel state known as *setting* within a reasonable length of time for the purpose for which it is used. When the material is applied to the human body, a reasonable length of time for the setting is between five minutes and a half hour. It is advantageous to be able to hasten or retard this setting time.

5. The setting point should be sufficiently high to allow the materials to set in a short period of time, yet not so high as to make it necessary to apply the materials at a temperature that would be harmful to living or dead tissue.

6. After setting, it should be firm. The material should have sufficient strength to hold firmly together under stress of a small amount of pressure so that it may be relieved of undercut surfaces.*

7. After setting, it should have enough elastic quality to take its original form after being stretched or bent away from an undercut surface.

8. It should be possible to use the material for making *waste molds*, *piece molds*, and *draw molds*.

9. It should be possible to pour or "paint" hot wax or resinous positive compositions into a set negative without destroying the mold or any part of it in doing so.

*Unfortunately, at the present time, the author knows of no plastic composition that can be applied over living tissue that forms a mold having strength enough to be roughly handled after setting. Agar compositions at best are weak as a comparison to good glue molds. A mold of vulcanized rubber-like qualities after setting would be ideal.

10. It should be suitable as a mold for the making of plaster of Paris casts.*

11. If the mold is composed of expensive ingredients, it should be possible to use such material as constitutes the mold over and over again, or the material should be so inexpensive that it can be thrown away after use.

After conducting over a hundred experiments with dozens of different ingredients during a period of three years, I have devised the following formula, which I believe will meet the necessary requirements for making hydrocolloidal molds of living and dead tissue:

| | |
|---|---|
| Agar | 4 ounces |
| Water | 100 ounces |
| Zinc oxide | 1 ounce |
| Tannic acid | 100 grains |
| Thymol | 20 grains |
| Glycerin | 1 ounce |
| Cellulose | ½ ounce |
| Cotton | 15 grains |
| Rubber cement | 4 ounces |

Rubber cement can be purchased from any art, stationery, or five and ten cent store. Such cement generally contains a small percentage of a resin such as damar or mastic. This does no harm to the mass. In fact, Poller includes it in his formulas according to his patents. It can also be made by dissolving a half ounce of caoutchouc (para-rubber) in twenty-five ounces of benzine. This takes about three days.

*Agar* is the basic substance; having the properties, when mixed with water, of melting into a complete liquid with no particles suspended, at about 100° C., and not returning to a gel or setting state until its temperature falls to about 42° C.

The *zinc oxide* increases the colloidal binding power of the agar. Ferric oxide may be used instead, if desired, in place of zinc oxide. Other metallic oxides may also be substituted, subject to experimentation. Zinc oxide causes the resulting mass to be white, while the ferric oxide causes it to be a dark red. In case the zinc oxide is used, the composition may be colored with any alcohol soluble dye to suit the taste of the caster.

The *tannic acid* is a tanning agent and acts somewhat as a separating influence toward gypsum.

The *cellulose* and *cotton* exert a mechanical influence in holding the particles of the mass together, increasing the internal friction of the mass both in the liquid and gel or set state.

The *thymol* and *glycerin* act as preservatives. The glycerin also serves to keep the composition moist. Methyl salicylate is also a good preservative.

*Unfortunately, a hydrocolloidal composition, such as one containing either agar or glue, is not a suitable molding material for the making of porcelain or terra cotta casts. The reason for this is the fact that these molds do not absorb water from the positive material. Plaster molds, however, do this when the porcelain or terra cotta slip is poured into them. Nor do the agar or glue molds have strength enough to allow the stiffer porcelain or terra cotta pastes to be forced or pressed into place.

The *para-rubber* in the rubber cement is supposed to increase the binding power of the mass and has some effect on its consistency, keeping it from flowing rapidly from a vertical surface.

*Directions for Mixing.*—Place the agar in about three-fourths of the water (75 ounces) (which should be room temperature) in a double boiler and stir vigorously. If the agar is powdered, it mixes readily; however, it should be stirred until all of the lumps have been absorbed. If the agar is in shreds, it will only absorb the water, not going into solution until heated. Heat is applied until the mass goes into complete solution.

The remaining one-fourth of the water is placed in a large-mouth bottle to which are added the oxide, cellulose cotton, thymol, tannic acid, and glycerin. The bottle is corked and the contents are shaken vigorously until they are thoroughly mixed. This shaking in the bottle separates the cellulose and cotton fibers as well as mixes the other ingredients.

After the agar and water have reached 100° C., or the boiling point, and have gone into complete solution, the oxide-cellulose or second mixture is poured slowly into the agar or first solution with constant stirring. When these mixed ingredients have again reached 100° C., the mass is removed a safe distance from the fire and the dissolved para-rubber or rubber cement, which is very inflammable, is poured slowly in while stirring. The mass bubbles up in evaporating the volatile liquid which was used to dissolve the rubber. It should not be replaced on the fire until the greater percentage of this chemical is removed by evaporation. Otherwise, it will ignite readily. After heating again to about 100° C. to drive off any remaining benzine, the mass is poured out into an enamel tray to cool. (If the manufacture of this product is done on a large scale, the benzine may be recovered by condensation.) On cooling, the composition will set, after which it is ground in a food chopper and allowed to stand until the water has evaporated from it to a point where none can be squeezed from the mass when a ball of it is gripped in the hand. On reheating the mass, it is ready for use. Of course, it is allowed to cool to a degree just about setting point before it is applied to living or dead tissue.

The composition is stored in wax covered, air tight containers to prevent drying out. Cylindrical cardboard boxes that have previously been dipped in wax serve well for this purpose.

*Requirements of a Positive Material.*—The best positive material for giving a lifelike reproduction of flesh is a composition having a resin and wax base. Plaster, papier mâché, and similar materials are inferior to wax as far as being "lifelike" for the casting of medical and biologic subjects. The positive composition can be made to be as durable as plaster, providing it is handled with the same care that is given a fine plaster casting.

The resin-wax composition used for the positive impression should possess the following qualities:

1. It should be possible to brush or "paint" the positive composition into a plaster or agar mold without its cracking or "lifting" from the surface of the mold.

2. Its melting point should be so high that it will not be affected by any degree of heat which is natural in the hottest weather. A cast, no matter how thin, should never droop as a candle often does under normal summer temperatures.

3. It must not crack or shrink to any great extent when subjected to the normal cold of winter.

4. It should not crack under sudden changes of temperature as when a thin layer of hot wax of a high melting point is poured into a cold mold.

5. The shrinkage of the wax should be so slight as not to cause cracks in it when it is shrinking against a rigid mold or after it has been mounted.

6. It should become set, yet remain pliable at a degree of heat low enough to be bent or formed with the hands without breaking or cracking the wax or fear of burning the hands, nor should the composition stick to the hands. This is advantageous as it often becomes necessary to bend slightly a positive impression into a more desirable shape. For example, it may be necessary to bend the positive to relieve it from some undercut surface on a rigid plaster mold. After the positive has been relieved of such an undercut, it can be rebent to its normal position.

Bearing these rigid requirements in mind, let us consider the published material on positive formulas:

Douglas[13] gives the following formula for the preparation of the positive wax impression:

"2 pounds white beeswax
1¾ pounds paraffin
1 lb. talcum powder
1 lb. prepared cornstarch
2 oz. yellow beeswax"

The talcum powder and cornstarch used in this formula do not increase the melting point of the combined waxes which is dangerously low (see table of waxes). While the cornstarch and talcum powder will decrease the cost and increase the bulk of the composition, I do not advise its use because it definitely increases the possibility of breakage or of crumbling which is alleviated somewhat by the low melting point and high cohesive qualities of the combined waxes used. (Had stearic acid been used, the possibility of crumbling would have been increased, due to the crystalline structure of this substance and its low cohesive qualities.)

It seems to me that the yellow beeswax is used in this formula more for the purpose of coloring than anything else. However, it may increase the pliability of the composition very slightly. If it is used for coloring only, it seems unnecessary, as pigments have to be added anyway to bring the wax to the desired color or shade. This formula cannot be brushed or painted into the mold successfully.

In a mimeograph sheet, issued by D. E. Ziskin,[14] of the School of Dental and Oral Surgery at Columbia University, the following formula is given:

"11 ounces white filtered paraffin (high fusing)
2 ounces light carnauba wax
½ ounce beeswax
¼ ounce dark carnauba wax"

This formula has a higher melting point than that of Douglas[13] and for this reason, I consider it more valuable. However, when we consider the three basic properties of each wax, i.e., expansion on heating, contraction on cooling, and elasticity, we find that carnauba has high cohesive and low adhesive properties. Therefore, it should be used only as a hardener and to raise the melting point out of danger of the heat of summer. For the above mentioned reasons, Ziskin's formula is very likely to crack when poured into a cold mold or subjected to violent changes in temperature. This could have been prevented by the addition of a resinous gum which would also increase the melting point. This formula cannot be painted into the mold successfully.

It seems that the small amount of dark carnauba wax was used mainly for coloring the composition, as it has the same melting point as the light carnauba. The same may be said of the beeswax providing it is yellow beeswax, for the quantities are so small that they change the cohesive and adhesive qualities to a minor degree. I believe in relying mainly on pigment for coloring rather than using dark waxes for this purpose, which do little more than complicate the formula.

In Golden's article,[7] which has been mentioned before under wax molds, he also considers positive compositions. For the wax mold, he naturally writes of substances which can be used without the application of heat. One of these is "a high grade of plaster having the advantages of ordinary plaster as to convenience in setting time and greater hardness than the artificial stones heretofore used. This plaster is known by the trade name of orthopedic plaster, or hydrocal 'B.'" It is made by calcinating dry gypsum rock under steam pressure which gives larger crystal structure than possible with ordinary plaster.[15] The normal setting time of this material ranges from twenty-five to fifty minutes. However, setting can be accelerated to a few minutes or retarded for several hours. As mentioned before, a resin-wax mixture gives a more lifelike reproduction.

Golden also considers "hominit and the other Poller positive materials." Of "hominit," he writes as follows:

"According to analytic tests, the product consists of: Carnauba wax, 73 per cent; rosin, 3 per cent; ash (composed of silica, lime and magnesia), 24 per cent.

"From the tests, appearance and color, no doubt a medium grade of No. 1 North country carnauba wax is used,[16] this giving the compound a brownish gray appearance due to impurities in this grade of wax. This color does not lend itself very readily for tinting, and its dark appearance, if not tinted, is deceptive in taking casts of the white race.

"The mixture could be benefited greatly by the use of No. 1 Flora pale yellow carnauba wax, which is the highest grade. By using this grade of

wax, a very light appearance should be assured which would allow the use of various tints to a finer degree, to give a natural lifelike skin coloring to the finished cast.

"By the addition of a small percentage of paraffin wax, the higher temperature wax preferably, ranging from 5 to 15 per cent, a milky tinge is given to the compound; which is an advantage in making death masks. This cloudy appearance can also be taken advantage of in the making of casts of children and women."

I disagree with Golden about the contents of "hominit" due to its very nature. The addition of only 3 per cent of a resin would not give the mass its characteristic bending qualities; nor would it be possible to brush the material into the mold. Furthermore, if it contained the amount of carnauba wax that Golden specifies, the mass would readily shrink and crack. Poller, himself, says in his British patent specifications:[17] "The colophony will always amount to more than half the resin mixture as otherwise the treatment with the brush is rendered more difficult." Poller continues to state that the carnauba should not exceed one-tenth of the amount of resin. He gives the following proportions:

| | |
|---|---|
| "Resin | 14 |
| Paraffin | 2 |
| (Clear) Carnauba | 1" |

I believe that these proportions are misleading in the fact that the formula may overestimate the amount of resin that is actually used in the average composition. It does not necessarily over- or understate the amount of carnauba wax used. I also believe that the amount of paraffin is underestimated in Poller's formula. As stated before: this is often done in patents to prevent commercialization.

In spite of the fact that Golden recommends the use of No. 1 Flora pale yellow carnauba wax and 5 to 15 per cent of high temperature paraffin wax, the composition is still very likely to be too yellow for the normal Caucasian skin. This can be eliminated by the use of a filler or color desensitizer such as talc, plaster, or kaolin (see author's formula). Any formula containing 73 per cent of carnauba wax will give a compound sure to shrink and crack on being poured in thin layers into a cold mold. It would be impossible to obtain a good cast by brushing a composition of this nature into any kind of mold. I do not believe that a really successful formula, which will meet the rigid requirements set down in this text, can be compounded from Golden's analysis of "hominit."

In further consideration of Poller's positive materials, such as "hominit," "celerit," and "granulit," there seems to be little difference in them other than color and melting points. Waxes and resins may be obtained in various shades of brown and yellow. These shades may be changed by the use of pigments, fillers, and color desensitizers. If one consults the melting points in the table of waxes and the table of resins, to say nothing of the facts laid down in this article under "*Compounding the Positive Formula*," one can

easily determine the materials to use to raise or lower the melting point of a composition. In such a manner the worker may compound formulas for specific purposes.

I believe that the major reason for producing positive compositions under different names lies in their commercial possibilities. I have yet to discover the advantages of starting out by using "hominit" for a thin coating to the mold, and then switching over to "celerit" to back up the cast or thicken it as Poller recommends. If one prepares his own mixture of a sufficiently high melting point and of the right color, he can use this one mixture for the entire thickness of his cast. Of course, some cloth or fiber material is used to strengthen the positive.

Toward the end of the article by Gross,[10] in the *Archives of Pathology*, mentioned before in this article, when we considered negative formulas, he recommends the use of plaster of Paris, sealing wax or paraffin for making the positive impression. I believe that there are far more suitable compositions for making the positive than any of the three that Gross recommends. The reasons for this belief are as follows: It is extremely difficult to obtain

TABLE I
TABLE OF WAXES

| NAME | MELTING POINT | COLOR | SOURCE |
|---|---|---|---|
| Bayberry | 45°-46° C.<br>113°-114.8° F. | Light green | Vegetable wax |
| Beeswax (natural)<br>Apis mellifera | 62°-64° C.<br>143.6°-147.2° F. | Yellowish brown | Insect wax |
| Beeswax (bleached)<br>Apis mellifera | 62°-64° C.<br>143.6°-147.2° F. | White | Insect wax |
| Candelilla | 68°-70° C.<br>154°-158° F. | Brownish to yellowish brown | Vegetable wax |
| Carnauba (light)<br>Corypha cerifera | 84° C.<br>183.2° F. | Light yellow | Vegetable non-glyceridic |
| Ceresin (ozokernite light) | 61°-78° C.<br>142°-172° F. | White | Mineral wax |
| Ceresin (ozokernite dark) | 61°-78° C.<br>142°-172° F. | Light yellow | Mineral wax |
| Chinese<br>(cocus cerifera) | About 81° C.<br>172° F. | Light yellow | Insect wax |
| Japan<br>Rhus succedsneum | 49° C.<br>128° F. | Light yellow | Vegetable fat |
| Montan | 80°-86° C.<br>176°-187° F. | Dark brown to white when bleached | |
| Paraffin* | 48°-62° C.<br>118°-144° F. | White<br>semitransparent | Mineral wax |
| Parawax† | 51°-58° C.<br>124°-137° F. | White<br>semitransparent | Mineral wax |
| Spermaceti | 42°-50° C.<br>107°-122° F. | Pearly white becomes yellow on prolonged exposure | Sperm whale wax |
| Stearic Acid<br>(Stearine) | 60°-65° C.<br>140°-149° F. | White<br>crystalline structure | Chemical |

*The melting point of paraffin varies from 48°C. to 62°C. It can be purchased for melting at the following temperatures: 48° to 50°, 50° to 52°, 52° to 54°, 56° to 58°, 60° to 62° C.

†"Parawax" is a trade name for paraffin manufactured by the Standard Oil Company of New Jersey. This wax is cheaper than some paraffins which melt at specific temperatures, and serves just as well for the purposes of wax casting.

a truly lifelike positive in plaster. Gypsum has a tendency to crumble, or "rot" as it is termed by casters, when incorporated with certain pigments. It does not have the texture or semi-translucency of skin or flesh as do wax and resin. In coloring the plaster of Paris positive, the color seems to lie on the surface rather than actually to appear as though it were incorporated in the cast.

TABLE II

TABLE OF RESINS

| | PROPERTIES | SPECIFIC GRAVITY | MELTING POINT | SOLUBILITY |
|---|---|---|---|---|
| Asphaltum | Black or brownish black substance intense in chromatic value | 1.0 to 1.68 | 100° C. | Five times its weight of naphtha and benzol Dissolved by alkalies and alkaline carbonates |
| Colophony or common rosin | Brittle, transparent pale amber to dark red brown | 1.045 to 1.085 | Softens at 70° C. to 80° C. semi-fluid in boiling water. 135° C. should not be exceeded | Methyl and amyl alcohol, acetone, ether, chloroform, carbon disulphide. Partial—at 60° C. dissolves slowly in equal weight of alcohol or glacial acetic acid. Petroleum spirit |
| Copal | White through shades of yellow, and red to brown and brownish black. Fossil resins have an oxidized crust containing mineral matter. Tree resin is odorless | 1.03 to 1.07 | 95° C. to 305° C. | 95% alcohol, oxidized turpentine, alkaline solutions |
| Dammar | Nodules—3 mm. to 4 cm. Powdery crust. Clear. Transparent. Odorless | 1.062 to 1.23 | Softens at 75° C. Syrupy at 100° C. Clear and thin at 150° C. | Benzol, chloroform, carbon disulphide, oil of turpentine<br>*Partial:* Methyl and ethyl alcohols, ether, acetone, glacial acetic acid |
| Mastic | Grains ovoid in shape. 0.5 cm. to 2 cm. Milky, dull yellow or greenish. Taste and smell like turnips | 1.04 to 1.07 | Chios 108° C. Other 103° C. | 80% chloral hydrate solution, ether, amyl alcohol, benzine<br>*Partial:* Methyl alcohol, acetone, acetic acid, chloroform, oil of turpentine |
| Sandarac | Form of grains 0.5 to 1.5 cm. Clear yellow drops. Surface has dusty appearance. Harder than mastic | Between 1.05 and 1.092 | Liquefies at 160° C. | Alcohol, ether, amyl alcohol, acetone, and several ethereal oils<br>*Partial:* Choloroform, turpentine. Fatty oils |
| Sheliac | Thin, translucent hard flakes. Varies in color from yellowish brown to black.<br>*Bleached or white shellac:* Prepared by dissolving crude lac in potash of soda, filtering and passing chlorine gas into the filtrate till all is precipitated | 1.139 | | Methyl and ethyl alcohols, acetic acid, alkalies, solution of borax<br>*Partial:* Ether, ethyl acetate, chloroform, acetone, carbon disulphide |

Sealing wax is expensive to buy or to prepare in comparison to other compositions. Furthermore its internal friction is too great for convenient use. In other words, it flows too sluggishly. It also has too much "gloss" for some cases. It is better to apply the "gloss" in the form of shellac or varnish to the area in mind than to try to remove the glossiness from the area where it is not wanted.

Paraffin, when used alone, has a melting point that is so low that the positive is likely to be destroyed by the heat of summer. It also has a high degree of gloss and is semi-transparent. I do not recommend the use of any of the substances in the form as mentioned by Gross for the making of casts of medical subjects.

In *Colorado Medicine*, there appeared an article by Mumey,[18] of Denver. This article recommends the use of plaster of Paris for making the molds of surgical specimens. This I do not advise. He also calls attention to the hydrocolloidal formula developed by Gross.

For his positive formula this author gives the following:

| | |
|---|---|
| "White beeswax | 8 ounces |
| Paraffin (high melting point 56° to 58°) | 8 ounces |
| Cornstarch | 6 ounces |
| Talcum powder | 6 ounces |
| Spermaceti | 1 ounce |

(Yellow beeswax may be added for color)

"The wax and paraffin are melted before adding the starch and powder."

In my opinion, this formula seems of little importance because of its low melting point and the fact that there is little difference in his three waxes (see table of waxes). Any one of them could have taken the place of all three without any material loss to the composition. Talcum powder and cornstarch in such proportions act as little more than fillers, cheapening the product and at the same time making it more crumbly.

It is interesting to notice that this author says: "The inside of the mold is coated with liquid albolene which aids in separating the wax positive from the plaster negative." I have never heard of this practice before in separating wax from plaster as most casters simply dip the plaster negative in water before "painting" or pouring the positive composition into it. The antagonistic qualities or immiscibility of oil (wax) and water create a perfect separating medium.

He also continues: "The positive wax cast is removed from the negative plaster cast by making saw cuts into the negative and carefully breaking it loose with a hammer." In my estimation, this is indeed a hazardous and clumsy method of casting in consideration of the fact that Mumey was familiar with the hydrocolloidal formula of Gross and the commercial product "negocoll." His formula cannot be "painted" into the mold.

In considering, as a whole, the positive formulas which have been mentioned in this article and published before, the following facts are evident:

1. They do not contain resin (except Poller's formula, in which I believe

the amount of resin is overestimated, and Golden's analysis of Poller's "hominit" which I believe is incorrect in the sense that the resin is underestimated).

2. They cannot be brushed successfully into the mold. (The only exception that may be to this is the formula of Poller.)

3. These formulas often contain more than one wax of about the same melting points, which I believe is unnecessary.

4. They often contain a small amount of an intense yellow or brownish yellow wax as a coloring agent. Color, I believe, should be applied to the positive formula in the form of color pigments.

5. Their melting points are dangerously low. I believe that the melting points should be higher.

It is for the reasons given above that I do not recommend the use of any of the formulas that are mentioned in this article, other than that humbly submitted by myself.

### ADVANTAGES OF BEING ABLE TO APPLY THE POSITIVE MATERIAL WITH A BRUSH

A positive resin-wax material which permits itself to be spread, or painted, instead of poured, into the mold offers decided advantages which may be enumerated as follows:

1. It eliminates the danger of the much dreaded borderlines, i.e., lines caused by the junction of hot and cold wax on a smooth surface. The hot and cold wax do not blend together, thereby forming a line where the waxes of different temperatures meet (Fig. 6-*A*).

2. The painting on of the positive permits a freeness of working without the necessity of having to lift heavy molds to turn them in various positions so the wax will flow evenly and of the same thickness over the entire mold. When large forms are to be poured, it otherwise would require a great outlay of physical strength and nerve-racking exertion to say nothing of the necessity of the assistance of helpmates to produce the cast.

3. It is far more economical to "paint" a mold than to pour one, as considerable wax may be lost or damaged beyond further use by slopping over the edges of the mold while it is being rotated, thereby soiling one's clothes and burning the hands. The composition may fall on the floor, worktable, or anywhere but in the place it should be.

4. It is possible to fill the separate pieces of a piece mold before they are put in place to insure their surfaces of being well and evenly covered. After they have been put in their proper places, the positive pieces may be cemented together by the application of more wax which may be poured, or painted, into the cracks between the positive pieces.

5. In painting with the positive substance rather than pouring it into the mold, it is possible to apply masses of different materials and colors next to one another in the same layer, or different colors in different layers, thereby producing the transparency of one color through which a second color may be seen beneath the first. One may spread on the mold a wax composition to resemble the skin in color and texture to the edges of a gaping,

moist, reddish wound that would ordinarily be glistening with high lights. This wound area of the cast can be painted in with a different material and color than that of the skin. While this second material may have a wax base, it can be so incorporated with other materials such as various gums to produce the desired glossy effect.

6. One may prepare a palette of an ordinary muffin pan, filling each cup with the positive wax material with which has been mixed a different color. The caster may then use this muffin pan as an artist does his palette in painting a canvas; the caster paints his mold instead. The thin applications of

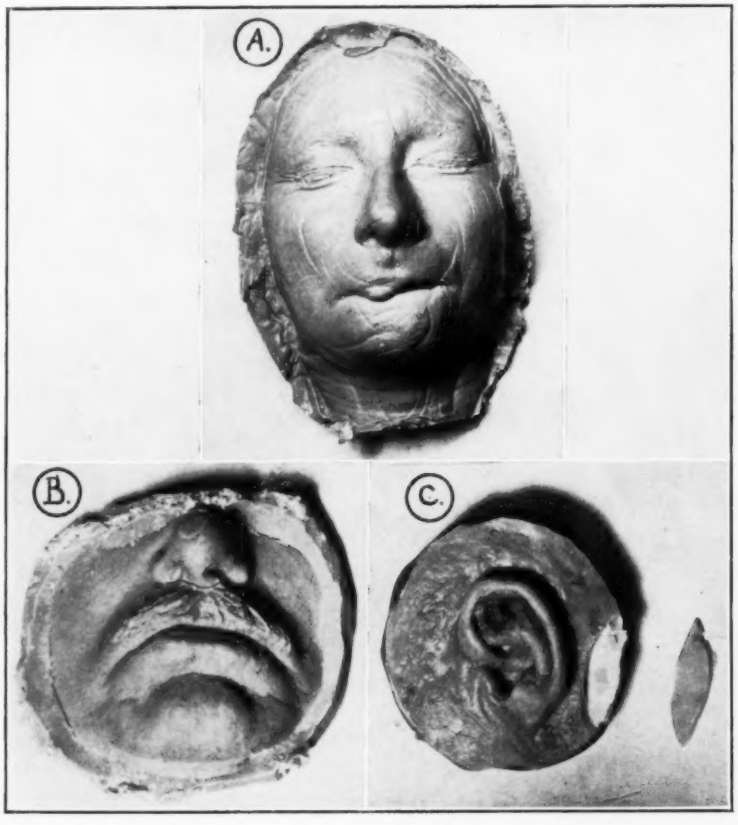


Fig. 6.—Mistakes of casting. *A*, Border or lap lines caused by an improper mixture of positive material being poured slowly into a cold mold. These lines are due to the fact that one layer of wax has set before an adjoining layer is poured. *B*, Cracks caused by shrinkage due to improper mixture of positive materials. Such cracks are caused by too much carnauba wax or not enough resin in the composition. *C*, Showing that gauze reinforcement was not completely saturated with hot wax before application. All fibrous materials should be dipped in hot wax before placing in the mold.

the various colors on this mold must be strengthened or backed up by a thicker layer of wax, then reinforced with gauze, cheesecloth or burlap fibers, or threads.

In such a manner, the small blue vessels that lie beneath the transparent skin can be incorporated into the cast in the most naturalistic manner. Another example for the use of color in this way is a typical hemorrhage under the skin. The fresh flowing blood possesses a different color from stagnant

blood. In such a manner vessels may be painted red on a thin coating of semi-transparent positive formula which has been applied to the mold. These vessel details are then backed with more composition and finally strengthened with gauze, cheesecloth, or burlap. When the cast is finally removed from the mold, the vessels may be seen as though they lie under the skin rather

Fig. 7.—Mounting. *A*, Side view of cast on submount. *B*, Notice that the submount takes the outline of the cast. *C*, Gauze is sealed to the edges of the cast which is applied with a hot electric tacking iron. *D*, The iron is used to seal the edges flat. *E*, This is continued all around the edges. Pleats are made where necessary. *F*, The gauze is finally turned under the submount and attached with carpet tacks. *G*, The submount is then screwed to the mount. *H*, Side view of the cast on the mount.

than painted on the surface as is so often done by the caster. It is interesting to note that *red* vessels under an ivory colored skin appear *blue* when viewed through the skin. This can be tested by placing a piece of ivory colored tracing paper over dark red marks drawn on white paper. These red marks will seem to be blue providing the right shade of tracing paper and the proper shade of red are used.

There are limitless possibilities for the specific use of a positive material that can be painted into the mold rather than having to be poured. In fact, they are too numerous to continue to mention here. However, they will suggest themselves as the worker begins the making of molds and casts.

*Compounding the Positive Formulas.*—Before preparing formulas for positive impressions, bear in mind that every substance in them must be there for a definite purpose. It seems that most casters mix a number of waxes together with great quantities of starch and a filler such as talc with little knowledge of the properties of the substances they use. White waxes having approximately the same melting points are likely to react in much the same way. Using more than one of these only complicates the formula and fools no one but the novice. Do not necessarily use a yellow wax for color; this is the work for color pigments to do, which have a far greater range of shades and tones than waxes. Furthermore, pigments are more dependable. In the best formulas it is often necessary to reduce the chromatic intensity of some waxes and resins rather than to add yellow wax to give them color. The following data are essential for the worker to bear in mind when he compounds formulas:

*Resin:* In spite of the fact that such work is often called "wax" casting, resin is the basic substance in the formulas that are the most simple to use and have most versatility. It gives the composition toughness, and the quality of bending without breaking, hardens more slowly and prevents warping and cracking to a great extent. Common rosin or colophony is generally used for this purpose because of its cheapness. Bleached rosin serves better where the chromatic intensity is to be kept low. *The resin content should amount to more than half of the mixture;* otherwise brushing the positive mixture into the mold is rendered difficult, leaving "lap lines" or borderlines. Such lines are caused where a hot mixture meets a cold or set mixture of the positive material, causing a line to appear at the junction of the hot and cold mixtures (Fig. 6-*A*).

*Balsams and Amylum:* Brittleness of resins can be reduced by the addition of balsams and amylum. The following balsams may be considered: Copaiba, Canada, Peruvian balsam, and gum elemi. As for the amylum, cornstarch and more particularly wheat starch may be considered. These starches tend to lower the chromatic intensity of the resin. *Only a small quantity,* about one-tenth of the weight of the resin, should be used. Balsams lower the melting point of the positive formulas. Amylum does not lower the melting point.

*Wax (Low Melting Point):* The viscosity, and viscidity, or internal friction, of a resin is reduced by the addition of a wax. A cheap wax, necessarily having a low melting point, is chosen for this purpose. Such waxes are: "parawax," paraffin, white beeswax, ceresin, and spermacetic. Here again the cheaper products ("parawax" and paraffin) are generally chosen.

*Wax (High Melting Point):* The melting point of a wax and resin composition can be raised by the addition of a wax having a high melting point. Such waxes are carnauba, montan, and Chinese. Carnauba (light) is generally chosen. *The amount of such a wax* that is used in the composition *should never be more than one-tenth of the weight of the resin;* otherwise shrinkage and brittleness of the entire composition will result.

*Zinc Oxide:* Zinc oxide may be used to render the resin harder and less sticky, also giving the composition a higher melting point. The weight of *the zinc oxide* should never be more than one-thirty-fifth of the weight of the resin. If more is added, a frothing up occurs and the mass will not remelt once it has set. It also will become as hard as stone, and crumbly.

*Fillers, Toners and Color Desensitizers:* Substances such as talc, plaster of Paris, kaolin, marble meal, whiting, powdered glass, and silver and gold or metallic powders have little effect on the melting point of positive resinous masses and serve only as fillers and to change the shade or color of the resulting compositions. *If such substances are added in too great quantities, the composition becomes brittle and crumbly.* Some white fillers, such as talc and plaster, are excellent for reducing the chromatic intensity of resins.

*Volatile or Drying Oils and Hydrocarbons:* Hydrocarbons such as benzine and drying oils such as turpentine, linseed, poppy oil, and naphtha may be used to lower, temporarily, the melting point of the mass (especially of hard waxes). This helps the resin to facilitate the bending of the positive impression after cooling, for removing it from undercuts in the rigid plaster negative. A few drops of benzine or some volatile oils eliminate the slight froth caused by the addition of zinc oxide to a formula. This slight addition does not harm the mass materially by lowering the melting point. The pot containing the composition should be moved a safe distance from the fire before adding such inflammable liquids. Continuous heating of the same positive mass is likely to raise the melting point and cause it to become brittle. This can be given its original properties by the addition of a small amount of drying oil. Some casters prefer to remove the cast from the mold after it has completely cooled. In such a case the addition of a drying oil is advantageous. However, it is often better to remove a cast, having a resin base from the mold when it is still slightly warm and pliable. In such a case the addition of a liquid to lower, temporarily, the melting point is unnecessary.

*Binders:* Casts may be strengthened by the use of cotton, hemp fibers, cheesecloth, gauze bandages, wool, burlap, and similar substances. Such materials are generally dipped in the hot positive composition and applied to the back of the cast after it has reached the thickness of about an eighth of an inch. These binders are used whether the positive is brushed or poured into the mold. This, of course, is done while the cast is still in the mold. According to Poller, "Northern" or Harras wool may be mixed directly with the positive formula for the purpose of binding the particles to one another. Such fibers do not "lump" together. If the cast is well supported with one or more of these binders, the chances of its being completely demolished on dropping a short distance to the floor is greatly reduced. In many cases, repairs are easily accomplished.

*The Author's Positive Formula.*—A good, simple and cheap (less than fifteen cents per pound) formula that I have devised to meet the rigid requirements set down further back in this text is as follows: This formula can be brushed or "painted" into the plaster or agar mold. It may be varied to suit specific requirements. "Parawax" is given before paraffin because it is cheaper. If a paraffin is used, choose one of a melting point similar to "parawax" (about 51° C. to 58° C.).

| | |
|---|---|
| "Parawax" (or paraffin) | 5 ounces |
| Rosin (colophony, bleached) | 10 ounces |
| Carnauba wax (light) | 1 ounce |
| Talc | 4 ounces |
| Zinc oxide | ¼ ounce |
| (Color to suit hue of subject)* | |

*Roehrig's transparent oil colors (for photo-tinting). If this is to be the skin color of a caucasian, it is only necessary to add a red pigment such as vermilion (blond) or rose madder (brunette). The red mixes with the yellow of the resin and carnauba, giving a "flesh tint."

The paraffin and carnauba wax are melted together until they are both in a liquid state. To prevent burning a double boiler is generally used for this purpose. The resin is then added and the composition stirred until the resin mixes with the wax into a homogeneous mass. The talc, which is used to reduce the chromatic intensity of the resin, is added with constant stirring, and then the zinc oxide while the stirring is continued. When the zinc oxide is added, a slight froth or foam may collect on the surface of the mixture. This may be expelled with a few drops of benzine which should not be added until the melting pot is a safe distance from the fire.

Color is added by placing a small bit in a tablespoon, then filling the spoon with some of the melted composition. The bottom of the bowl of a teaspoon is used to mix the color into the small quantity of wax in the tablespoon. After the color is well mixed in the tablespoon, both spoons are used to stir the pot of composition, thus permeating it with color. This process is gone through with because the color, when placed directly into the composition, does not mix readily with it. Bear in mind that color should be used sparingly because it is difficult to reduce the color intensity by the addition of a filler without harming the entire structure of the positive mass. Wax-resin compositions decrease in chromo value when the mass cools. If it is so desired, this formula can be mixed as a medium in small quantities with many shades and colors. The caster can paint the inside of his mold with the color he desires. Of course, this does not mean that more color should not be added to the cast after removal from the mold. In fact, it should be touched up with oil, tempera, or water colors, to make it more realistic.

Let us consider why certain substances were used in the formula that has just been given:

1. The "parawax" or paraffin was used to reduce the internal friction or sluggishness of the resin base.

2. The resin, among other reasons mentioned before, made it possible to "paint" or brush the composition into the mold using any small paint or varnish brush for the purpose.

3. The carnauba wax raised the melting point out of danger of summer heat.

4. The talc reduced the chromatic intensity of the yellow of the resin and carnauba, thereby making it possible to obtain a true flesh tint in the entire composition.

5. The zinc oxide reduced the stickiness of the resin and helped raise the melting point of the completely cooled mass.

6. The benzine eliminated the foam, thereby keeping it from appearing on the surface. This is not always necessary but is good insurance against a bubble-infested surface on the cast.

This formula may be varied to suit particular purposes and climates. For example: In cold climates the amount of carnauba wax may be reduced. In hot climates it may be increased. Different fillers may be used to create the effect of metal, wood, stone, and marble, according to what is desired.

A formula better suitable for pouring should contain more wax and less resin than the formula given above as the internal friction of the above formula is too great.

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# AN IMPROVED CONGO RED TEST FOR AMYLOIDOSIS*

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KEITH, Rowntree and Geraghty[1] introduced a method for the determination of blood volume by means of vital red. This dye was injected intravenously and normally did not leave the blood stream in four to six minutes. By that time the dye was found to be distributed evenly in the circulating blood. Keith and others have shown that the dye does not enter the blood cells and can be found quantitatively in the serum.

Griesbach,[2] in his study of blood volume, injected 10 c.c. of a 1 per cent Congo red solution and found a uniform distribution of the dye in the serum after four minutes. The dye began to leave the blood ten minutes after injection; however, after one hour 90 per cent was still in the circulation of normal subjects, and after twenty-four hours no further traces of Congo red could be found in the serum.

Bennhold[3] found that in persons with amyloid tissues, Congo red leaves the blood stream either in toto or in large part within an hour. His method was to inject intravenously 10 c.c. of a 1 per cent Congo red solution and withdraw two specimens of blood, the first at four minutes, and the next, one hour after injection. The blood was then centrifuged and comparison made on the serum by means of the colorimeter. The four-minute specimen was used as the standard from which the amount of absorption in one hour was determined. Using this technic he found a disappearance of 11 to 29 per cent of the dye from the blood stream of normal persons in one hour, whereas if 60 per cent or more of the dye disappeared, amyloidosis was indicated. When the absorption fell between 40 and 60 per cent, he considered it indicative either of nephrosis or amyloidosis.

The Congo red test for amyloidosis used in the past is satisfactory, providing the serum obtained is unhemolyzed. Extreme precautions are necessary, otherwise the clinician can rely on values only where the absorption from the blood stream is complete, and even with these precautions there can be no assurance that hemolysis has not occurred unless the specimen is examined with the spectroscope. An excessive amount of lipoids in the serum gives a turbidity that interferes with colorimetric readings.

These disadvantages can be overcome by the use of ethyl alcohol which precipitates the proteins and dissolves the Congo red, giving a clear solution that can be read with the colorimeter.

Method.—The test is carried out on the patient in a postabsorptive state. Ten cubic centimeters of a 1 per cent Congo red solution are injected into a vein and four minutes later about 10 c.c. of blood are withdrawn from another vein. The second sample of blood is taken

*From the Laboratory Division, Sea View Hospital.
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one hour after injection. Here it might be advisable for the tubes to stand about two hours to permit the clots to retract. The tubes are next centrifuged at a moderate speed (3,000 r.p.m.) for ten minutes, and the serum carefully decanted. Two cubic centimeters of serum are pipetted into 15 c.c. graduated conical test tubes and made up to 10 c.c. with 95 per cent ethyl alcohol. The tubes are corked, shaken for thirty seconds, and centrifuged at a high speed for ten minutes. (Absolute alcohol may be used, although it is not essential.) The clear supernatant fluids are poured into colorimeter cups and readings made on the colorimeter. The readings are best when the four-minute specimen is set at 20 mm. The calculation is

$$100 - \left(\frac{4 \text{ min. reading}}{1 \text{ hour reading}} \times 100\right) = \text{per cent absorption.}$$

The solution remains unchanged indefinitely when corked. Lipoidal serum gives a very clear supernatant alcoholic solution.

Any hemoglobin that may be present is precipitated by the alcohol, and the Congo red determined quantitatively. Readings before and after alcohol precipitation checked very closely, except where hemolysis was known to occur. In the table below can be seen comparisons of the same specimens by both methods:

TABLE I

| SERUM READ DIRECTLY PER CENT ABSORPTION | AFTER ALCOHOL PRECIPITATION PER CENT ABSORPTION | DIFFERENCE PER CENT |
|---|---|---|
| 11 | 12 | +1 |
| 15 | 12 | −3 |
| 36 | 30 | −6 |
| 10 | 10 | 0 |
| 31 | 33 | +2 |

This method can be applied only to serum. Experiments on whole blood with added Congo red do not give quantitative recoveries when the dye is extracted with alcohol. It seems that some Congo red is carried down with the precipitated hemoglobin, which if present in small amounts in the serum does not affect the readings. Congo red added to serum is extracted quantitatively with alcohol.

The test has been satisfactorily applied in the authors' laboratory. One case is especially of interest. A patient was admitted into the hospital with a recent history of amyloidosis. He still showed clinical signs of the disease, a four-plus albumin, and Congo red in the urine after administration of the dye. Examination of the serum directly showed no absorption, in fact the one-hour specimen was slightly stronger in color than the one of four minutes. The addition of alcohol to the serum showed the absorption to be complete. Through use of this method it was possible to correlate the laboratory and clinical findings.

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# IMPROVED PROCEDURES FOR ESTIMATING BISMUTH IN BODY FLUIDS AND TISSUES*

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THE demand for reliable methods for the quantitative estimation of minute quantities of bismuth, which may exist in body fluids and tissues, has increased since the advent of bismuth in antisyphilitic treatment. Experiences in this laboratory during a period of eight years with a method, which has been steadily improved and simplified, have resulted in routine procedures which may be of interest and value to others. These procedures are applicable to all body fluids and tissues and have been used successfully in thousands of estimations. They are based upon unpublished data and some published data scattered through several publications from this laboratory. Many details, included in the original description,[1] are omitted here as they are no longer essential for most users of bismuth methods. The essential technical details and steps, including some typical results, follow:

*Equipment.*—Kjeldahl flasks (Pyrex) 300 c.c.; Folin sugar tubes (Pyrex), calibrated to 4 c.c. and 25 c.c.; two 500 c.c. burets, one for $HNO_3$ and one for $H_2SO_4$; two 200 c.c. volumetric flasks for sulphite and iodide solutions, fitted with 5 c.c. pipettes; Dubosecq colorimeter; gas hot plates; system of Pyrex glass manifolds and water tower connected with a suction device for the removal of fumes from the Kjeldahl flasks, 30 of which are connected in 3 groups of 10 each; crucibles (porcelain or silica), capacity 50 c.c.; electric furnace (Multiple Electric Unit Furnace, Type 66, No. 39, 461, New Duty Electric Co.); steam or water-bath.

*Solutions.—Standard bismuth solution* (Leonard): 0.223 gm. $Bi_2O_3$ (analyzed, C.P., and dried to constant weight at 110° C.) is dissolved in a small amount of $HNO_3$ and made up to 2,000 c.c.; 1 c.c. of solution = 0.1 mg. Bismuth. The standards for 0.001, 0.002, 0.005, and 0.01 mg. are set at 90 mm. on the colorimeter; the standard for 0.02 mg. is set at 50 mm. (using 20 c.c. cup); the standard for 0.05 mg. is set at 30 mm.; the standards for 0.1 and 0.2 mg. are set at 20 mm. *Potassium iodide solution:* 2.4 gm. of chemically pure (analyzed) KI are dissolved in 200 c.c. distilled water; 5 c.c. of this solution are used in each determination. The solution is discarded if it becomes discolored. *Acidified sodium sulphite solution:* 1 gm. ($Na_2SO_3$) is dissolved in 150 c.c. distilled water, then 0.8 c.c. concentrated $H_2SO_4$ are added and sufficient water to make 200 c.c.; 4 c.c. of this solution are used in each determination. This solution must be prepared fresh daily. Superoxol (concentrated $H_2O_2$); concentrated sulphuric acid, C.P.; concentrated nitric acid, C.P.; caprylic alcohol; $KNO_3$ (dry, C.P., analyzed). *Permanent standards:*

0.5000 gm. potassium chromate (C.P.) in 1,500 c.c. water = 0.20 mg. Bi
0.0750 gm. potassium chromate (C.P.) in 490 c.c. water = 0.100 mg. Bi
0.7800 gm. potassium chromate (C.P.) in 1,000 c.c. water = 0.050 mg. Bi
0.0500 gm. potassium chromate (C.P.) in 1,500 c.c. water = 0.020 mg. Bi
0.0174 gm. potassium chromate (C.P.) in 1,000 c.c. water = 0.010 mg. Bi
0.0077 gm. potassium chromate (C.P.) in 1,000 c.c. water = 0.005 mg. Bi
0.0066 gm. potassium chromate (C.P.) in 1,000 c.c. water = 0.002 mg. Bi
0.0064 gm. potassium chromate (C.P.) in 1,000 c.c. water = 0.001 mg. Bi

*From the Department of Pharmacology, Stanford University School of Medicine.
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*Check of permanent standards:* Introduce 10 c.c. of distilled water and add 5 c.c. of concentrated sulphuric acid each into four 25 c.c. calibrated sugar tubes. Add enough of the standard bismuth solution to contain 0.2, 0.1, 0.05, and 0.02 mg. of bismuth in different tubes. Cool the tubes and make up to the 25 c.c. mark, using 4 c.c. of sulphite water. Now add 5 c.c. of potassium iodide solution and match the permanent standards.

*Urine.*—The volume of urine to be used depends on the bismuth content. A 50 c.c. aliquot portion of a 24-hour sample is generally used. Human and animal (rabbit) urines are treated the same. To the urine contained in a Kjeldahl flask add a drop or two of caprylic alcohol, 5 c.c. of concentrated sulphuric acid, 10 c.c. of concentrated nitric acid, and 5 c.c. of superoxol. The flask is connected with the system of manifolds with a loose gooseneck tube, and combustion is started at once.

Boil on the hot plate until the nitric acid fumes have disappeared and the organic material is charred. Allow to cool partially and then further additions of nitric acid may be made without sputtering. If the acid (about 5 c.c.) is carefully added, frequent additions may be made even while the flask is on the hot plate. Superoxol is also added in the same way from time to time until the residue is water-clear. The removal of the last traces of oxides of nitrogen, which, by liberation of iodine from potassium iodide, affect the color of the determination, is achieved by adding 2 or 3 c.c. of superoxol to the residue and heating until the white fumes of $SO_3$ are formed.

*Colorimetric Estimation.*—Transfer the residue, which should be at least 4 c.c., from the Kjeldahl flask to a 25 c.c. Folin sugar tube, using a few small rinsings of water so as to bring the total volume in the tube to approximately 21 c.c. Allow to cool and add 4 c.c. of acidified sulphite solution and bring the total volume to the 25 c.c. mark, with distilled water, if necessary. Five cubic centimeters of potassium iodide solution are added and the whole centrifuged. The clear supernatant fluid is estimated for its bismuth content as soon as possible, using the permanent standards. In case less than 4 c.c. of concentrated sulphuric acid remain in the flask after digestion, more should be added; if there is more, it should be boiled down to approximately 4 c.c. If for any reason nitric acid drips back into the flask while being cooled, yellow to brownish discoloration of the digest from liberation of iodine may occur, but this is a rare occurrence; additional boiling discharges the color, in the event of such an occurrence.

*Feces and Tissues.*—Normal animal tissues are reported to contain from 0.002 to 0.005 mg. of bismuth in 1 gm. material, or at least give a color equivalent to this amount of bismuth. We have found the equivalent of 0.0009 mg. Bi in amounts of "blank" tissue up to 25 gm., plus the necessary reagents and treatment, and always subtract this quantity from a tissue analysis. The following procedure is used with blood, liver, muscle, kidney and feces; cerebrospinal fluid is treated like urine.

The crucible (porcelain or silica) is first carefully washed in concentrated hydrochloric acid and rinsed in distilled water (to be Bi-free). Charge the crucible with 5 gm. of feces or up to 10 gm. of tissue (liver, blood, brain, kidney, or muscle) and heat on a water-bath until dry. Keep tissues covered. Six to 8 hours of drying are sufficient. (Brain and kidneys from rats and other small animals are used in quantities of 1 or 1.5 gm.) Place the crucible and contents in an electric furnace and heat gradually from 725 to 900° C. (pyrometer). Too rapid heating causes sputtering. If a sliding asbestos tray is introduced into the furnace, several crucibles may be placed on this tray and the whole gradually admitted into the furnace, which has been preheated. Considerable time can be saved in this way. Heat for twelve to fifteen hours, or overnight. The crucibles are inspected at the end of this period and any carbon still remaining may be readily oxidized by adding approximately 0.5 gm. powdered potassium nitrate and heating for another hour. Several additions of potassium nitrate may be necessary, especially with brain and liver. When a white ash is obtained, remove the crucibles from the furnace, cool, and add 5 c.c. concentrated $H_2SO_4$ to each, making certain that the sides are washed down well with the acid. Let stand for one hour. Transfer, by washing, the contents of each crucible into a Kjeldahl flask of 300 c.c. capacity, and add 10 c.c. $HNO_3$ and continue wet oxidation on a hot plate, as with urine. Rarely more than a single addition of nitric acid is necessary. The addition of 5 c.c. of superoxol greatly facilitates oxidation. From here on the estimation proceeds as with urine. Occasionally heating

of rabbit feces results in considerable loss of bismuth, so with rabbit feces an excess of $KNO_3$ is used. The addition of almost any salt, such as $Na_2CO_3$, NaCl, or $KNO_3$, before commencing the incineration, will prevent loss of bismuth. Explanation for this is not at hand, but it may be the presence of additional flux which helps to avoid loss of Bi. With blood, liver, and kidney tissue, the hot digest commonly appears yellowish in color which is presumably due to iron, but on cooling becomes colorless and the change does not affect the estimation.

Typical examples of recoveries of bismuth added to different tissues are presented in the table, including means and ranges of colorimeter readings for reagents and tissues; the blank value for subtraction in all analyses being 0.0009 mg. Bi. Quantities down to 0.001 mg. Bi can be accurately estimated. Quantities of the order of 0.001 mg., such as may occur in cerebrospinal fluid, have been checked qualitatively and quantitatively with a spectrograph.

TYPICAL EXAMPLES OF RECOVERED BISMUTH ADDED TO VARIOUS BODY FLUIDS AND TISSUES*

| | COLORIMETER READINGS | QUANTITIES BISMUTH ADDED | | |
|---|---|---|---|---|
| | | MG. BI | COLORIMETER READING | PER CENT RECOVERY |
| | Mean Range | | Mean | |
| Reagents | 98 (75-120) | 0 | | 0 |
| Reagents and $FeSO_4$ (0.5 gm.) | 88 | 0 | | 0 |
| Blood | 101 (87-110) | 0.001 | 94 | 96 |
| Brain | 99 (85-110) | 0.002 | 91 | 99 |
| Muscle | 100 (84-110) | 0.005 | 91 | 99 |
| Liver | 98 (81-110) | 0.010 | 92 | 98 |
| Kidney | 94 (81-110) | 1.000 | 21† | 95 |
| Feces (rabbit) | 86 (76-110) | 5.000 | 23† | 87 |
| Feces (human) | 105 (101-110) | 0.010 | 96 | 94 |

*The compounds used were sodium iodobismuthite ($Na_2BiI_5$) and sodium bismuthate ($NaBiO_3$).

†Different standard-level.

## CONCLUSION

Procedures for the quantitative estimation of bismuth in body fluids and tissues are outlined.

## REFERENCE

1. Hanzlik, P. J., and Mehrtens, H. G.: Clinical Excretion of Bismuth; Bismuth Metal, Arch. Dermat. & Syph. 22: 483, 1930.

# *DEPARTMENT OF REVIEWS AND ABSTRACTS*

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**RENAL DISORDERS, Rapid Quantitative Method for Examining the Urine in, Gibbons, H.** Arch. Int. Med. **54:** 758, 1934.

*Enumeration of Formed Elements.*—The patient is instructed to go without fluids after breakfast the day before the test. In the evening of the day, on retiring, he empties the bladder, discards the urine and notes the time of doing so. The next morning he passes urine into a collecting bottle, again notes the time and brings the specimen to the laboratory for examination.

The volume of the collected urine is measured in a 1,000 c.c. graduate cylinder. The urine is well mixed. A volume equal to the number of hundred cubic centimeters in the total urine (i.e. 1 per cent of the total) is placed in a 15 c.c. graduate centrifuge tube. The urine is then centrifugated until all the elements are deposited at the bottom of the tube. The greater part of the supernatant urine is now pipetted off with a capillary pipette until it is reduced to the proper volume for calculation. If the centrifugated portion is from an eleven or twelve hour specimen, the volume must be 0.5 c.c., if from a nine to eleven hour specimen, the volume must be 0.4 c.c., and if from a seven to nine hour specimen, 0.33 c.c. The sediment is then mixed with the aid of a pipette, and a drop is transferred to a ruled blood counting chamber. The average number of elements in 0.1 c.mm. (one small square) is then counted. This number is divided by 2. The resulting figure is the number of million elements excreted by the patient in twelve hours. In making the count the low power objective should be used for counting casts, and the high power for the cells. This method is applicable to all specimens of urine, and gives the same picture for all similar renal lesions.

The number of elements in a twelve hour specimen is calculated by the Addis method as follows:

$$\frac{\text{Number of elements in 1 small square} \times \text{volume of specimen}}{0.0001 \text{ c.c.}} \times \frac{\text{12 hour volume}}{\text{Volume centrifugated}} = \text{number of elements excreted in twelve hours.}$$

In these fractions it is possible to have all except two items constant. The two variable items are: (1) the number of elements counted in one small square, and (2) the twelve hour volume. It is possible to make the quotient of the fraction $\frac{\text{Twelve hour Volume}}{\text{Volume centrifugated}}$ constant by having the denominator (volume centrifugated) always equal to 1 per cent of the numerator (twelve hour volume). When this is done the only variable item influencing the result is the number of elements counted in one small square. Furthermore, when, instead of a twelve hour specimen the patient brings an eight, nine, or ten or eleven hour specimen, it is necessary to correct the volume to twelve hours. But the calculation for this can be avoided by taking the centrifugated sediment up in, say, eight-twelfths of 0.5 c.c., or 0.33 c.c, for an eight hour specimen; nine-twelfths or 0.37 c.c., for a nine hour specimen; ten-twelfths, or 0.41 c.c., for a ten hour specimen, or eleven-twelfths, or 0.45 c.c. for an eleven hour specimen. Because the error in the final result is small, it is unnecessary to correct closer than a two hour difference. Thus, a seven to nine hour volume is taken up in 0.33 c.c., and a nine to eleven hour volume is taken up in 0.4 c.c., and the different times can be so arranged that the number of elements counted when divided by 2 will give the result in millions of elements per twelve hour specimen.

For example: 1. Given a twelve hour specimen of urine, the volume is 400 c.c. and 12 red blood cells are counted in one of the nine squares of the counting chamber. Therefore, according to the formula:

$$\frac{12 \times 0.5 \text{ c.c.} \times 400 \text{ c.c.}}{0.0001 \text{ c.c.} \times 4 \text{ c.c.}} = 6{,}000{,}000 \text{ red blood cells.}$$

2. Given a ten hour specimen, the volume is 333 c.c., with the precipitate taken up in 0.4 c.c. (note that the volume does not change in the calculation) and 12 red blood cells are counted in one of the nine squares.

Therefore:

$$\frac{12 \times 0.5 \times 333 \text{ c.c.}}{0.0001 \times 3.3} = 6{,}000{,}000 \text{ red blood cells.}$$

*Estimation of Urine Protein.*—In order to know the rate of output of albumin, it is necessary to know the quantity excreted over a definite period of time. Addis calculated the excretion of protein of normal and diseased kidneys for twelve hour periods. By simple mathematics, the volume of urine excreted during twelve hours can be estimated from the volumes collected in from eight to eleven hours. Then, with 1 c.c. of urine, a calculation of the percentage of protein present is made, and from this information the excretion of protein in twelve hours is determined. For this estimation the simple and rapid procedure for measuring albumin described by Lashmet and Newburgh has been adapted. The turbidity, or cloud, produced by adding sulphosalicylic acid to the urine containing protein is compared with that of a standard turbidity solution representing a known protein content.

*Stock Solution.*—To about 200 c.c. of distilled water in a 500 c.c. volumetric flask, 50 c.c. of a tenth-normal solution of sodium hydroxide, U.S.P., and 8 gm. of cupric sulphate, U.S.P. (hydrous) are added. The volume of 500 c.c. is made with distilled water.

*Standard Solution.*—After shaking the stock solution vigorously to insure uniform suspension, exactly 4 c.c. of this mixture is transferred to a 100 c.c. graduate cylinder and tap water is added up to the 50 c.c. mark. The turbidity of this mixture is the same as that produced by a 0.1 per cent solution of protein, 0.1 gm. per one hundred cubic centimeters, when sulphosalicylic acid is used. This solution can be kept in a flask for use when wanted. The turbidity does not change on standing. It must, however, be shaken several times before using.

In order to determine the amount of protein in the urine, 1 c.c. of the centrifugated urine is placed in a 100 c.c. graduate cylinder, and a 2 per cent solution of sulphosalicylic acid is added to bring the volume up to 25 c.c. The standard solution is placed in a second 100 c.c. cylinder for comparison. If the turbidity of the mixture is the same as that of the standard solution the urine contains 0.1 per cent protein. If the turbidity is greater than that of the standard solution, the mixture should be diluted with tap water until it compares with the standard. Correction for the dilution is done by multiplying 0.1 per cent by the number of times the 25 c.c. volume has been diluted; then the corrected figure is multiplied by the number of cubic centimeters of urine excreted in twelve hours. The result is the number of grams of protein excreted in twelve hours. If the turbidity of the mixture is less than that of the standard solution, urine should be added until the same turbidity as that of the standard solution is obtained. The twelve hour volume is then divided by the number of cubic centimeters of urine added and multiplied by 0.1 per cent. Not more than 5 c.c. of urine should be added to the reagent; if no cloud is formed with this amount, the albumin content of the urine is negligible. This is a rough estimate of the rate of excretion of protein, and is applicable for practical use in determining the type or the progress of a renal lesion.

**WHOOPING COUGH, Blood Picture in Experimental, Inaba, I., and Inamori, S.** Am. J. Dis. Child. **48**: 1193, 1934.

The authors succeeded in producing experimental whooping cough by means of infection in eight young monkeys and six puppies. Except in one mild case, leucocytosis and lymphocytosis were observed. The symptom appeared after from three to four weeks of

incubation, often before the development of paroxysmal cough, and therefore is not a mechanical effect of cough but a biologic reaction caused by infection with the pathogenic bacillus. After the infection and before the appearance of lymphocytosis, no leucopenia could be proved. For the diagnosis of whooping cough and the determination of its stage, it is much more profitable to depend on the absolute number of lymphocytes than on the total leucocyte count and the percentage of lymphocytes, especially when complications are present.

**SPINAL FLUID, Diagnosis in Spinal Fluid Contaminated by Blood: The "Bloody Tap," Soloman, P.** New England J. M. **212:** 55, 1935.

The following criteria are useful:

| | "Bloody Tap" | Previous Subarachnoid Bleeding |
|---|---|---|
| Homogenous admixture of blood | 0 | + |
| Clot formation | + | 0 |
| Xanthachromia in supernatant fluid | 0 | + |

The following proportion enables a leucocyte count in bloody fluid:

R.B.C. b = peripheral R.B.C. count
W.B.C. b = peripheral W.B.C. count
R.B.C. f = fluid red cell count
W.B.C. f = fluid leucocyte count
W = leucocytes originally in fluid

Then: $W = \text{W.B.C. f} - \frac{\text{W.B.C. b}}{\text{R.B.C. b}} \times \text{R.B.C. f}$

Example: Leucocyte count on peripheral blood = 20,000
W.B.C. count in spinal fluid = 260
R.B.C. count in spinal fluid = 25,000

Then: $W = 260 - \frac{20{,}000}{5{,}000{,}000} \times 25{,}000 = 260 - 100 = 160$

Calculation of Protein Content: P
P = original protein content of fluid
R.B.C. b = red cell count of patient
Pb = serum protein
H = hematocrit value of blood
Pf = protein content of supernatant fluid

For most purposes Pb can be taken as 7 gm. per cent; H as 43 per cent; R.B.C. b as 5,100,000

Then $P = Pf - \frac{\text{R.B.C. f}}{\text{R.B.C. b}} \times Pb \times (1-H)$

or, with the average values given above: P = Pf – 0.0008 R.B.C. f.

The following dicta represent good approximation.

1. To obtain the original white cell count in the spinal fluid, subtract 1 leucocyte for every 500 red cells.

2. To obtain the original protein content subtract 4 mg. per 100 c.c. for every 5,000 red cells (or, roughly, 1 mg. per 1,000 red cells).

3. Sugar and chloride values are not appreciably affected by contaminating blood.

4. The colloidal gold reaction is not affected unless the red cells are over 5,000. Even 25,000 produce only a few 2's in the curve.

5. A positive reaction in bloody spinal fluid is significant only when the blood Wasserman is negative.

**POLIOMYELITIS, A Successful Method for Vaccination Against, Kolmer, J. A., Klugh, G. F., and Rule, A. M.** J. A. M. A. **104:** 456, 1935.

Twenty-five children varying in age from eight months to fifteen years have been given from one to three injections of poliomyelitis vaccine at the request or with the consent of parents.

Fifteen of these children were without antibody in serum neutralization tests before immunization and eleven, or 75 per cent, showed large amounts of antibody in the blood one week after the last dose of vaccine.

Ten of the children showed the presence of antiviral antibody in the blood before immunization, but all have shown a considerable increase of this antibody after vaccination, so that antibody production occurred in twenty-one, or 84 per cent, of the group of twenty-five children.

None of the twenty-five children have shown the slightest ill effects from the vaccine.

Mild local reactions were produced at the sites of subcutaneous injection, with occasional slight elevation of temperature and slight leucocytosis subsiding within forty-eight hours.

The dosage for children from one to three years of age has been 0.25, 0.5, and 0.5 c.c. at weekly intervals; for children from four to ten years, 0.5, 0.5, and 1.0 c.c.; for children from eleven to fifteen years, 0.5, 1, and 1 or 2 c.c. For adults the dosage recommended is 0.5, 1, and 2 c.c.

The vaccine is prepared of spinal cord only, as brain contains too small amounts of virus. But the spinal cord of a single monkey will furnish about 150 c.c. of vaccine, which is sufficient for the immunization of from forty to seventy-five children, depending on age and dosage.

It is likely that the maximum antibody response may be obtained by giving the injection every ten days instead of weekly.

Antibody production, however, appears to be fairly rapid, as three susceptible children developed antibody in the blood within four days after the first injection of vaccine and one monkey was found completely and a second partially immune seventy-two hours after the subcutaneous injection of 0.5 c.c. of vaccine per animal on intracerebral inoculations of large amounts of virus.

The vaccine does not appear to produce a demonstrable "negative phase" of increased susceptibility, after injection.

The vaccine is a 4 per cent suspension of spinal cords of monkeys developing poliomyelitis after intracerebral inoculation with a remote passage strain of virus, in a 1 per cent sterile solution of sodium ricinoleate prepared as previously described. The virus is attenuated and the vaccine regarded as entirely safe for the immunization of human beings not only because prepared of remote passage virus which probably has lost in infectivity for human beings but likewise because of attenuation by sodium ricinoleate, the route of administration and the injection of a small first dose.

The amount of antibody produced by immunization is comparable to that found in the blood in natural immunity and is believed to be sufficient for affording protection against acute anterior poliomyelitis.

The antibody present in the serums of vaccinated children has successfully neutralized human virus from the 1934 California epidemic.

The duration of the immunity following vaccination is unknown but has lasted for more than three years in vaccinated monkeys.

It is believed that the vaccine is now ready for vaccination of human beings and especially children against poliomyelitis and particularly during epidemics.

**CARCINOMA, Early Cutaneous, Sutton, R. L.** J. A. M. A. **104:** 433, 1935.

Sutton formulates the following postulates.

1. (a) Many skin cancers begin as de novo lesions. (b) The earliest visible lesion in these cases is a circumscribed, scaly, epithelial newgrowth.

2. (a) The structure of many minute, scaly, epithelial newgrowths is such that it is reasonable to presume that, if not interrupted, they would become obvious carcinomas. (b) It is reasonable to believe that such lesions are in fact early carcinomas. (c) If a lesion has a structure not compatible with a likelihood of its being early carcinoma, it might be called precancerous. But it would be impossible to predict that such a lesion might develop a structure, if uninterrupted, such that it would be properly called carcinoma.

3. (a) It is impossible to determine at what point in its natural history a cancerous lesion was not cancerous. (b) It is reasonable to believe that cancer is cancer from the start.

4. The concept of precancerosis is indecisive and undefinable. It groups unrelated conditions, which may or may not be early cancer. Its acceptance entails an insoluble problem of a dividing line between cancer and noncancer, as well as an insoluble problem of statistical assay of lesions that are strictly individual.

5. (a) A lesion may be cancerous independently of its size and rate of growth. (b) Cancer is primarily an epithelial disease. (c) A cancer consists of mutated somatic cells. (d) The earliest visible manifestations are circumscribed, dyskeratotic lesions which microscopically are composed of polymorphous epithelial cells that proliferate, keratinize and undergo mitosis in an abnormal manner. (e) Malignancy depends on a balance between the proliferative capacities of its cells and the control or resistance of the host. (f) One tumor may contain several kinds of cells as a result of mutation following on mutation.

6. Early cancerous lesions are readily destroyed and cured. In suspecting all early lesions and destroying them, one would prevent the development of all late lesions such as might become incurable.

**RENAL FUNCTION: Sodium Ferrocyanide as a Clinical Test for Glomerular Efficiency, Stieglitz, E. J., and Knight, A. A.** J. A. M. A. **103**: 1760, 1934.

The method follows: 0.5 gm. of hydrated salt, or about 0.25 gm. of the anhydrous salt is prepared in sterile ampules (those for the study were supplied by the Abbott Laboratories).

The contents of one ampule completely dissolved in water is intravenously injected and urine specimens collected at 30, 60, 120 and 180 minutes after the injection.

The Titration: The titration is carried out with a quantitative copper sulphate solution made to contain 0.4 per cent of copper sulphate (0.004 gm. per cubic centimeter). The content of copper is determined exactly by electrolysis. Before titration the specimens are acidified with concentrated sulphuric acid. Copper ferrocyanide is an insoluble red salt but is less insoluble than the well-known Prussian blue. This fact is used in determining the end-point of the titration. Drops of concentrated ferric chloride solution are placed on a tile and as the titration proceeds a drop of the unknown solution is placed in contact with the ferric chloride. If free ferrocyanide is present an immediate formation of Prussian blue occurs; if all the ferrocyanide has been precipitated as cupric ferrocyanide, a distinct delay occurs in the appearance of this blue color on the tile. The more insoluble ferri-ferrocyanide slowly replaces the slightly more soluble cupric ferrocyanide. The end-point therefore is read when the appearance of blue is delayed appreciably (five seconds), and the number of cubic centimeters of copper sulphate required is noted.

If hematuria exists during the period of ferrocyanide elimination the method of titration must be modified, for the sulphuric acid apparently liberates sufficient ferric ions from the hemoglobin to precipitate Prussian blue and thus prevent the formation of the red cupric ferrocyanide. This difficulty is readily avoided by determining the volume of the specimen of urine, bringing it to a quick boil, which precipitates the protein, and then filtering it. A measured quantity of this filtrate is then acidified with concentrated sulphuric acid and titrated in the usual way, the results being corrected to the original volume of the specimen.

The following conclusions were reached:

Sodium ferrocyanide, in doses of 0.5 gm. of the hydrated salt (0.25 gm. of anhydrous sodium ferrocyanide) has been used in more than 100 individuals for the purpose of studying the glomerular efficiency.

It has proved entirely nontoxic in this dosage, when slowly administered intravenously.

The normal secretion curve is characteristic and shows but relatively little spread of variation. This variation is less than that of phenolsulphonphthalein excretion.

So far as is known, ferrocyanide salts are excreted solely by way of the glomeruli.

In hypertensive arterial disease the excretion of ferrocyanide is considerably retarded, much more so than the phenolsulphonphthalein elimination.

In congestive heart failure both the phenolsulphonphthalein and the ferrocyanide elimination are impaired.

In known glomerulonephritis the excretion of sodium ferrocyanide is either nil in severe cases or else very much reduced.

This test offers notable potentialities of clinical usefulness, since it is specific for the glomeruli, is safe, is simple of execution, and is quite constant in the normal.

In physiologic investigations of the mechanism of renal secretion the procedure should also prove of considerable value.

Much further work is required before the full significance and import of these studies are understood. The authors hope that this preliminary report may encourage others to extend and amplify this work; it has been their object merely to present a new method and to point out some of its potentialities.

**ANEMIA of Prematurity, Josephs, H. W.** Am. J. Dis. Child. **48**: 1237, 1934.

A group of premature babies has been studied during the first three or four months of life, for the purpose of determining, if possible, the mechanism of the physiologic anemia of prematurity. Importance has been attached to the response of the reticulocytes and especially its relation to the administration of iron. From the study of the reticulocytes it was concluded that there is no basis for the idea that the fall in red cells and hemoglobin was dependent on hypoplasia in any strict anatomic sense. There was a period of failure to react to the administration of iron that lasted for from six to ten weeks after birth, the duration of the period depending largely on the degree of prematurity. After this early period of nonreactivity there followed a short transition period with delayed response, after which administration of iron was followed by a prompt response of the reticulocytes and a rise in red cells and hemoglobin. There is no reason to believe that lack of stores of iron plays any part in the development or persistence of the anemia before the end of the third month. Thereafter, the rise in red cells without a corresponding rise in hemoglobin in untreated infants indicates the possibility that lack of stores of iron has become an important factor. From the results reported here one cannot conclude that a low percentage of hemoglobin is in itself harmful, although it probably is indicative of a condition that renders the infant more likely to succumb to infection. In such cases, although iron may raise the hemoglobin content, transfusion must still remain the method of choice in treating the condition as a whole. The evidence from this study does not permit the conclusion that liver is necessary as an adjunct to iron, though in individual cases it may prove of benefit. As in the case of iron, liver given alone in the early period was ineffective, but given later was followed by a response which differed in no way from that following the administration of iron. Copper had no demonstrable effect.

# *REVIEWS*

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

## Laboratory Diagnosis*

THOSE familiar with the first edition of Osgood and Haskin's book on Laboratory Diagnosis will not be surprised to find that a second edition of this useful and practical text has become necessary.

The present volume, dedicated to the memory of the coauthor of the first edition, has been extensively and thoroughly revised by Dr. Osgood and embodies much new material representing the newer advances in the field of laboratory medicine.

Among the more important changes are discussions of the blood urea clearance, the insulin coefficient, the determination of blood bromides, the Friedman test, the galactose tolerance test, the heterophile antibody reaction, medicolegal applications of blood grouping, and the quantitative determination of proteins in spinal and other body fluids.

Widely known for his studies in hematology, Dr. Osgood presents the results of many investigations relating to hematologic technic as well as concerned with normal values.

Despite the extensive changes throughout the text generally, the original plan of the book has not been altered.

Clinical pathologists have long decried the misuse of laboratory procedures which, after all, if they are to fulfill their true function, must be regarded, not as diagnostic tests, but as phases in the examination of the patient requiring not only intelligent application but, particularly, intelligent interpretation.

The first section of this book, therefore, is devoted to a discussion of the application of laboratory procedures in the study of disease and suggests the answers to the crucial questions: What are the laboratory studies most likely to be informative in a particular problem? When and how often should they be utilized? What is the clinical significance of their results?

The second part of the book is devoted to technical methods.

The style throughout is clear, simple, and without ambiguity. The illustrations are equally good.

A feature of the book is the double index. An index by diseases enables the practitioner to determine in a particular condition what laboratory methods are of material aid and refers him to a discussion of their clinical significance and interpretation.

A general index makes the contents of the book readily available.

It may be said with confidence that this book constitutes a practical, comprehensive, and authoritative text destined to become a standard reference.

It may be recommended without reserve to student, practitioner, and laboratory worker alike.

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## Failure of the Circulation†

THIS is a book for which the physician at large has been waiting, whether he knows it or not. Certainly it presents in a clear, interesting, and comprehensive fashion, a well-conceived and equally well-carried out concept of the hemodynamic aspects of cardiac and circulatory disease as considered from both the clinical and physiologic aspects.

*A Textbook of Laboratory Diagnosis. By Edwin E. Osgood, M.D., Assistant Professor of Medicine and Biochemistry, University of Oregon, School of Medicine. Cloth, pp. 585, 27 figures, 10 colored plates. P. Blakiston's Son & Co., Philadelphia, Pa.

†Failure of the Circulation. By Tinsley R. Harrison, M.D., Associate Professor of Medicine, Vanderbilt University School of Medicine. Cloth, pp. 396, 60 figures. Williams & Wilkins Co., Baltimore, Md.

The book is divided into five main sections: I. The Hypokinetic Syndrome, in which the clinical picture as well as the underlying causes of cardiac insufficiency are considered; II. The Hyperkinetic Syndrome, which discusses the overactive heart and cardiac neurosis; III. The Dyskinetic Syndrome in which the inefficient cardio-circulatory system is discussed under these subheadings: A. General Clinical Considerations; B. Dynamics of Congestive Heart Failure; C. The Major Phenomena of Congestive Heart Failure; D. The Minor Phenomena of Congestive Heart Failure; E. The Prognosis and Treatment of Congestive Failure.

Part IV discusses Mixed Types of Circulatory Failure and Part V Failure of the Coronary Circulation.

In the last analysis, there are but four main problems confronting the physician in the consideration of the cardiovascular system of a patient. These, as stated by Maher are: (1) What caused the cardiac disease? (2) What is the structural lesion? (3) How is the physiology changed? and (4) What are the patient's limitations?

In their elucidation Dr. Harrison's book will be of great value and assistance. It may be recommended without reserve as an excellent and valuable contribution.

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## The Harvey Lectures*

IN THIS volume, the twenty-ninth series of *The Harvey Lectures,* the following topics are authoritatively discussed: Typhus and Rocky Mountain Spotted Fever in the United States, by Dr. R. E. Dyer; The Potential Energies of Oxidation-Reduction Systems and Their Biochemical Significance, Dr. W. Mansfield Clark; Heteroplastic Grafting in Embryology, by Dr. Ross G. Harrison; The Estrogenic Substances, by Dr. E. A. Doisy; The Clinical Application of Some Recent Knowledge of the Biliary Tract and of the Pancreas, by Dr. Evarts A. Graham; The Significance of Morbid Processes in the Fetus, by Dr. George L. Streeter; Filterable Viruses With Special Reference to Psittacosis, by Dr. Thomas M. Rivers; and The Nervous Mechanism of Cardio-Vascular Control, by Dr. Detlev W. Bronk.

As always these volumes constitute a welcome and valuable addition to the physician's library.

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## Quarterly Bulletin of The Health Organization, League of Nations†

THE Special Number issued by The Health Organization of the League of Nations comprises the report of the Permanent Commission on Biological Standardization on proposed international standards for gas gangrene antitoxin, antipneumococcus serum, Types I and II, and Staphylococcus Antitoxin.

The issue of December, 1934, contains a lengthy report of the Demographic Setting of Infant Mortality, The Fifth Analytical Review of Reports from Pasteur Institutes on the Results of Anti Rabies Treatment, A Brief Guide to the Varieties of Anopheles Maculopennis, A Report on Milk Hygiene in the Department of Meurthe-et-Moselle, and Current Notes on the Work of The Health Organization.

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## A Textbook of Histology‡

IN THIS volume Cowdry presents histology in not only an interesting and vivid fashion, but also in such a manner that it can be applied to the problems of everyday medical practice.

As he remarks in the Preface, histology cannot be regarded as in the past it has been to some extent, as an isolated subject but must be accepted as an integral part of the study

*The Harvey Lectures. Delivered Under the Auspices of The Harvey Society of New York under the patronage of the New York Academy of Medicine. Cloth, pp. 262. Williams & Wilkins Co., Baltimore, Md.

†Quarterly Bulletin of The Health Organization, League of Nations, III:4: December, 1934, and Special Number, January, 1935.

‡A Textbook of Histology: Functional Significance of Cells and Intercellular Substances. By E. V. Cowdry, Professor of Cytology, School of Medicine, Washington University, St. Louis. Cloth, pp. 503, 242 engravings. Lea and Febiger, Philadelphia, Pa.

of the body, and as having an interlocking relationship with normal function, physiology, on the one hand, and abnormal function, pathology, on the other.

In considering the properties and functions of cells it must be recognized that there is no such thing as a generalized kind of cell existing alone. All are influenced by their heredity, the fluid about them, and the other cells with which they are associated.

The aim of this volume, therefore, is to build up a conception of cells and primary tissues in the many environments in which they normally exist and to stress the organs and systems in such a manner as to relate structure and function in the whole body, which is itself a physiologic unit.

The principal sections into which the book is divided are: Water, The Essential Vital Medium, The Blood, The Principal Integrator, Absorptive Drainage into the Blood, Chemical Integration by Endocrine Products in the Blood Stream, Intake of Water, Nutriment, Accessory Food Factors, and the Removal of Waste, Oxygen Consumption and Carbon Dioxide Elimination, Rapid Neural Integration, Architectural Structure, Perpetration of the Race, Unification, Protection, and Adjustment.

The practical conception of histology and its excellent presentation in the text result in a volume of interest and use to student, practitioner, and pathologist alike to whom it may be recommended without reserve.

The format and illustrations are in keeping with the volumes of this publisher.

## The Spleen and Resistance*

THAT the mechanisms governing the development of natural resistance are of great and obvious importance has long been recognized and that the spleen is in some way concerned with these phenomena has long been known. The exact nature of the function of this "enigmatic organ," as Marine well calls it, yet remains to be elucidated but the researches presented in this volume constitute a definite advance.

Beginning with a brief chapter on anatomic considerations the authors then consider in some detail the pathological changes in the spleen in a variety of infections, acute, chronic and virus diseases as well; the spleen as an organ of macrophage tissue; its relation to antibody formation; the effect of splenectomy on natural resistance; the relation of the spleen to acquired resistance in latent infections; compensatory changes following splenectomy; the significance of depression in resistance following splenectomy; and, finally, the variability of the effect of splenectomy in animals and its significance in the interpretation of the physiology of the spleen.

The survey thus presented embodies not only a comprehensive and critical review of the literature but also the results of the experimental investigations of the authors and, in all, constitutes, perhaps, the first systematic presentation on the subject.

A final résumé together with a bibliography and subject index enhance the value of the book.

## The Story of Medicine in the Middle Ages†

THAT interest in the history of medicine has not been as acute and as general among physicians as it should be, may have been accounted for by the relative dearth, until recent years, of volumes such as this. For it will be received enthusiastically not only by those now interested in medical history but without doubt will arouse equal interest in those who may have been dismayed by more ponderous tomes.

Dr. Riesman has chosen for his subject the history of medicine during the period commonly spoken of as "the Dark Ages" for, curiously enough, as he says in his Preface, there is more familiarity with the mythology of Greece and the history of ancient Rome than with the period called "the Middle Ages" although this period is so much nearer to us.

*The Spleen and Resistance. By David Perla, M.D., Associate Pathologist, Montefiore Hospital, and Jessie Marmarston, M.D., Associate in Pathology, Cornell Medical School. With a Foreword by David Marine, M.D. Cloth, pp. 170. The Williams & Wilkins Co., Baltimore, Md.

†The Story of Medicine in the Middle Ages. By David Riesman, M.D., Professor of the History of Medicine, University of Pennsylvania. Cloth, pp. 402, 79 figures. Paul B. Hoeber, New York.

The account which he gives of the development of medical lore and practice and of those associated with it is not only authentic and comprehensive, but written with charm and sympathetic understanding. It is an easily flowing story filled with incident, light, color, and vivid pictures in which the actors appear, not as shadowy phantoms dimly seen through the murky mists of long dead centuries, but as living worthies sharply etched against the panorama of their times.

It is a book which one suspects gave the author as much enjoyment in the writing as the reader will derive from its perusal.

Here are tales not only of individual struggles and achievement, but of the times in which they occurred; of astrology and astrologers, alchemy and alchemists; the surgeons and barbers of Paris; medieval baths and sanitation; medieval epidemics; leprosy, the sweating sickness and St. Anthony's Fire; the King's evil and epilepsy—all of which left their mark upon medicine and its development and here they are seen as vivid actualities.

Both author and publisher are to be congratulated upon a worth-while book, one which can be read and reread and the reader, perhaps, is to be congratulated most of all that it has been made available.

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## International Medical Annual*

NOW in its fifty-third consecutive year, the *International Medical Annual* needs no introduction for it has been the desk companion of innumerable physicians ever since the first volume appeared.

As before, the book begins with a review of the year's work in the treatment of disease, followed by the characteristic terse yet ample summaries of the literature on disease and its treatment. As usual, the subjects are alphabetically arranged, which with the comprehensive index makes the subject matter readily accessible.

As an authoritative reference to recent advances the volume can be recommended without reserve.

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## Laboratory Manual†

THIS small volume, prepared for the guidance of students and staff members, presents the routine methods in use in Peiping Union Medical College.

Intended as a guide to laboratory methods the text is restricted entirely to technic. Despite its size the book is quite comprehensive. Directions are detailed and clearly given and the book may be accepted as a safe guide for the methods described.

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## The Kidney in Health and Disease‡

THIS volume is the outcome of a symposium on the structure and function of the kidney in health and disease conducted at the University of Minnesota a few years ago under the direction of Dr. Hilding Berglund, then Professor of Medicine in that institution.

The contributions then made, amplified and revised to cover recent advances, constitute the material now presented and represent probably the most comprehensive survey of this subject yet published under one cover.

Each of the forty-one contributors is an accepted authority in the field whereof he writes.

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*International Medical Annual. Edited by H. Letheby Tidy and A. Rendle Short. Cloth, pp. 522, 64 plates. William Wood & Co., Baltimore, Md.

†Laboratory Manual of the Department of Bacteriology and Immunology of Peiping Union Medical College. By C. E. Lim. Ed. 2. Cloth, pp. 190. Kwang Yung Press, Peiping, China.

‡The Kidney in Health and Disease. Edited by Hilding Berglund, M.D. formerly Chief, Department of Medicine, University of Minnesota, and Grace Medes, Ph.D., Research Biochemist, Lankenau Hospital Research Institute. Cloth, pp. 774, 163 figures. Lea and Febiger, Philadelphia, Pa.

The volume is divided into six main sections: I. Anatomy and Physiology; II. Clinical Aspects of Renal Functions; III. Bright's Disease and Other Pathologic Renal Conditions; IV. Albuminuria and Edema; V. Ocular Changes in Bright's Disease; and VI. Clinical Aspects of Bright's Disease.

As these headings indicate, the book is not a presentation of abstract theories of interest only to the research worker, but is replete with material of real interest and practical value to the practitioner, the pathologist and, indeed, to all concerned with the kidney in its relation to disease.

The sections on the Clinical Aspects of Renal Function, Bright's Disease, Albuminuria and Edema, and the Clinical Aspects of Bright's Disease will be of great interest and value to the practitioner.

It is obvious that the last word upon the functions and diseases of the kidney has not yet been written and that there are many aspects of this subject which must still be the subject of controversial discussion. Nevertheless the present contribution furnishes a comprehensive and well-planned survey of the status quo at present.

There is, naturally, some slight unevenness in the assembling of such varied material from numerous contributors but the editors deserve much credit for the care with which the material has been arranged and correlated.

The format and the illustrations are excellent.

An author's index and general index make the material readily accessible.

This book deserves a place in the physician's library.

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## The Woman Asks the Doctor*

THIS little book is addressed to the laity, especially to women who, as the author remarks in his Preface, "are eager to know something of the significance of the remarkable cyclical phenomena which characterize their sex, particularly as these have for centuries been enshrouded in a mantle of mysticism."

The value of accurate information on this subject is apparent and yet, unfortunately, it is all too often disseminated by dubious routes and is often garbled and misleading.

The doctor and the gynecologist, who should be the proper avenues for instruction in these matters, should welcome Dr. Novak's book as covering the subject fully and yet simply.

The scope of the volume is apparent from the chapter headings: What Is Femaleness; Superstition and Folklore of Menstruation; The Reproductive Apparatus of Women; The Cause and Significance of Menstruation; The Glands as Related to Female Functions; The Beginning of Womanhood (Puberty); Characteristics of Normal Menstruation; The "Change of Life" (Menopause); Hygiene of Menstruation; Woman as an Egg-Producer; With Remarks on the So-Called "Safe Period"; Some Common Disorders of Menstruation; Sterility in Women; Leucorrhea; Cancer, The Arch-Enemy of Women; A Little About the Sex-Life of Women.

This book may be safely recommended to the audience to whom it is addressed.

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## Annals of the Pickett-Thomson Laboratory†

IN this volume the authors present a comprehensive survey concerned with the literature of influenza with special reference to the complications and sequelae, bacteriology of influenzal pneumonia, pathology, epidemiological data, prevention, and treatment.

The thoroughness with which the task has been carried out is exemplified by the bibliography embracing 4,500 papers.

Those fortunate enough to have seen the previous volumes published by these workers will find the present one of equal interest and value.

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*The Woman Asks the Doctor. By Emil Novak. Associate in Gynecology, Johns Hopkins Medical School. Cloth, pp. 189, 11 figures. The Williams and Wilkins Co., Baltimore, Md.

†Annals of the Pickett-Thomson Research Laboratory: Vol. X (Part II), Influenza. By D. and R. Thomson. Paper, pp. 1557. The Williams and Wilkins Co., Baltimore, Md.

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## EDITORIAL

### Tissue Penetration

THERE has recently been described a curious property of certain substances in promoting the spread of themselves or of other substances from a central focus into the tissues. This applies to various tissues but most especially to the subcutaneous tissues. The most recent work on this line has evolved from the observation of Duran-Reynals that testicle extract had the power of diffusing through tissues.

Claude has continued these investigations and has found that a diazo compound of sulphanilic acid possesses the same diffusing property. Probably the most interesting part of this observation, however, is that it not only diffuses itself but also other particulate matter. Thus, if it is given intracutaneously into the skin of a rabbit along with India ink, the India ink diffuses out through the tissues many times farther than when India ink is given in the same way but with physiologic saline instead. Diazo compounds of anilin and of arsanilic acid reacted likewise.

Claude next carried his investigation into the diazotizing of the aromatic amino acids, tyrosine, and histidine.

The simpler compounds had been quite irritating to the skin but these newer compounds were nonirritating and produced even greater spread. He finally diazotized protein and found that these azoproteins penetrated the skin and carried the India ink to even greater distances. These also were nonirritating.

Apparently any protein could be coupled with the diazo compound with the resulting azoprotein exhibiting this penetrating ability. The proteins used were serum, egg albumen, and gelatin. The degree of penetration varied and appeared to vary depending upon the content of tyrosine and histidine in the protein. The diazotizing process appeared to require tyrosine or histidine or some other cyclic grouping. The activity of the azoprotein in tissue penetration appears to be dependent upon its content of these particular amino acids. The spreading power of azoprotein appears to depend on the number of diazo groups attached to the protein molecule.

A normal spread of the India ink indicator covers an area of about 6.8 sq. cm. whether the additional solution be Ringer's solution, normal horse serum, or sulphanilic acid. When the last of these has been diazotized, the spread averaged 22.3 sq. cm. The spread with azogelatin was 31.7 sq. cm. with azoserum about 70, and with azoalbumin 137 sq. cm.

This ability of azotized protein to penetrate long distances through the subcutaneous tissues and to carry other matter with them holds promise of being a very interesting phenomenon, possibly with some potentialities for practical application. The simpler compounds, particularly azotized analin products are highly bactericidal. This suggests the possibility of the introduction and distribution of a germicide in subcutaneous infection. On the contrary we must recall as stated above, that testicular extract appeared to enhance bacterial infection, probably by this same method of promoting spread. Claude remarked "So far there is no evidence that azoproteins and testicle extract have a common mechanism of action, although their ultimate effect on tissue permeability may be the same."

Unfortunately the higher compounds, especially the azoproteins have lost completely their bactericidal effect in vitro. But, if such compounds will carry India ink with them they might also carry germicidal substances in solution, provided such a substance could be developed which would be highly toxic for bacteria and relatively nontoxic for tissues.

One might also conceive of the possibility of the development of a method of therapeutic penetration of tumors.

At present none of these possibilities are in the offing and attention is called to Claude's observation merely as a curious and interesting phenomenon which merits further study.

REFERENCE

Claude, Albert: Spreading Property of Azoproteins in the Dermis, J. Exper. Med. 62: 229, 1935.

*W. T. V.*